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LIFE-CYCLE PARAMETERS OF *TISBE BATTAGLIAI* (COPEPODA: HARPACTICOIDA) AS INDICATORS OF CHRONIC TOXICITY

by

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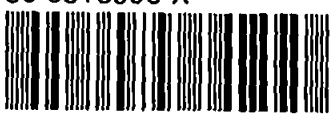

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ABSTRACT

There is growing concern about the fate and biological effects of chemical contaminants in the marine environment. In the United Kingdom, the present ability to detect the potential long-term effects of contaminants is limited by the lack of suitable laboratory methods for measuring chronic toxicity. The harpacticoid copepod *Tisbe battagliai* was selected as a candidate test organism and a suite of chronic toxicity test methods was developed for measuring the effect of chemical contaminants on individual copepods (postembryonic development, reproduction and life-table analysis) and populations of *T. battagliai.* The development of chronic test methods proceeded alongside investigations of the influence of key environmental variables (temperature and food availability) on the biology of this species. These investigations provided a valuable insight into the potential importance of these environmental factors for influencing the development of populations of *T. battagliai* in the field, and helped to define the optimum conditions for the culture and chronic toxicity testing of this species in the laboratory. The methods were further evaluated using pentachlorophenol (PCP) as a reference toxicant and the aim was to investigate the potential interaction between toxicant (PCP), environmental factors (temperature and food availability), and their effects on the population dynamics of *T. battagliai*.

In summary, results showed that temperature, and food quantity and quality, were important determinants of population dynamics. There were significant interactions between the chosen environmental variables (e.g. temperature), PCP, and subsequent biological effects on *Tisbe battagliai*, and results highlighted some important differences in toxicity testing approaches based on the measurement of individuals and populations of copepods. Established laboratory toxicity test procedures do not take account of the degree of complexity in the natural environment and this underlines the difficulty in extrapolating from laboratory results to the field situation.

In conclusion, the project was successful in its primary objective of developing a suite of techniques that can be used to measure the potential chronic toxicity of chemical contaminants in the marine environment. The methods using *Tisbe battagliai* are relatively simple to perform, are amenable to standardisation and provide relatively cost-effective measurements of chronic toxicity. The test methods can be used to provide chronic toxicity data but, more importantly, they can be used to address some of the current limitations associated with single species laboratory tests. For example, used in conjunction with key environmental variables, the methods provide a greater understanding of the potential interaction between contaminants and abiotic variables, thereby, improving the extrapolation of laboratory results to the field situation. The ability to carry out measurements on individual and populations of *T. battagliai* provides a valuable insight into the predictive links between effects at different levels of biological organisation.

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Lastly, I wish to thank my family and friends, without whose support, I would not have got this far.

AUTHORS DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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A programme of advanced study was undertaken which included the development of algal and copepod culture techniques and preliminary studies to develop toxicity test methods (acute and short-term chronic methods). These techniques were applied in a sea-going workshop on biological effects techniques sponsored by the International Council for the Exploration of the Sea (ICES) and the Intergovernmental Oceanographic Commission (IOC), held at the Alfred-Wegener Institute for Polar and Marine Research (AWI) in Bremerhaven, Germany, from 12 to 30 March 1990.

Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultation purposes and several papers prepared for publication.

Publications:

1 Hutchinson, T.H. and Williams, T.D. (1989). The use of sheepshead minnow (*Cyprinodon variegatus*) and a benthic copepod (*Tisbe battagliai*) in short-term tests for estimating the chronic toxicity of industrial effluents. *Hydrobiologia* **188/189**, 567-572.

2 Williams, T.D. (1992). Survival and development of copepod larvae *Tisbe battagliai* in surface microlayer, water and sediment elutriates from the German Bight. *Marine Ecology Progress Series* **91**, 221-228.

3 Williams, T.D., Hutchinson, T.H., Roberts, G.C. and Coleman, C.A. (1993). The assessment of industrial effluent toxicity using aquatic micro-organisms, copepods and fish. In: *Proceedings 2nd European Conference on Ecotoxicology*, Amsterdam, 11-15th May 1992, *Science of the Total Environment, Supplement 1993*, pp 1129-1141.

4 Hutchinson, T.H., Williams, T.D. and Eales, G.J. (1994). Toxicity of cadmium, hexavalent chromium and copper to marine fish larvae (*Cyprinodon variegatus*) and copepods (*Tisbe battagliai*). *Marine Environmental Research* **38**, 275-290.

5 Williams, T.D. and Jones, M.B. (1994). Effects of temperature and food quantity on postembryonic development of *Tisbe battagliai* (Copepoda: Harpacticoida). *Journal of Experimental Marine Biology and Ecology* **183**, 283-298.

6 Williams, T.D. and Jones, M.B. (in preparation). Effects of temperature and food quantity on the reproduction of *Tisbe battagliai* (Copepoda: Harpacticoida).

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Presentations and Conferences Attended:

1 Williams, T.D. (1992). Acute and chronic toxicity tests using life stages of the marine copepod *Tisbe battagliai*. Oral presentation at the *13th Annual Conference of the Society of Environmental Toxicology and Chemistry*, Cincinnati, Ohio, USA, November 1992.

2 Williams, T.D., Hutchinson, T.H., Roberts, G.C. and Coleman, C.A. (1993). The assessment of industrial effluent toxicity using aquatic micro-organisms, copepods and fish. Poster presentation at the *Second European Conference on Ecotoxicology*, Amsterdam, The Netherlands, May 1992.

3 Williams, T.D. and Jones, M.B. (1994). Effects of temperature and food quantity on postembryonic development of *Tisbe battagliai* (Copepoda: Harpacticoida). Poster presentation at the *Symposium on the Developmental Biology of Marine Organisms*, organised by the Marine Biological Association and the Society for Experimental Biology, Plymouth, April 1993.

4 Williams, T.D. and Jones, M.B. (1994). Temperature and food effects on development and reproduction in *Tisbe battagliai* (Copepoda: Harpacticoida). Poster presentation at the *International Council for the Exploration of the Sea (ICES) Symposium on Zooplankton Production*, Plymouth, August 1994.

5 Williams, T.D. (1994). The development of test methods using life stages of the marine copepod *Tisbe battagliai*. Poster presentation at the *15th Annual Conference of the Society of Environmental Toxicology and Chemistry*, Denver, Colorado, USA, November 1994.

6 Williams, T.D. and Jones, M.B. (1995). Effects of temperature and food quantity on the reproduction of *Tisbe battagliai* (Copepoda: Harpacticoida). Oral presentation at the *European Marine Biology Symposium*, University of Southampton, September 1995.

7 Williams, T.D. (1996). The development of test methods using life stages of the marine copepod *Tisbe battagliai*. Oral presentation at the *Society of Environmental Toxicology and Chemistry (UK Branch) Conference on Direct Toxicity Assessment*, University of Luton, July 1996.

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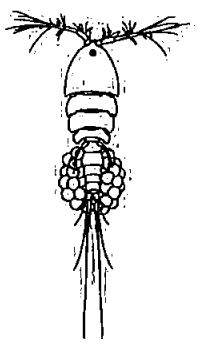


...the more you look the more you see.

—PETER GRANT,
Ecology and Evolution of
Darwin's Finches

This thesis is dedicated to my family.

CHAPTER 1

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

The principal objective of this research was to develop laboratory toxicity test procedures for measuring the potential long-term effect of chemical contaminants to marine organisms. The stimulus for this research is explained, together with the scientific issues concerning the selection of candidate test species. Key concerns regarding current toxicity test approaches are considered and attempts to resolve some of these issues within the framework of test method development are described.

1.1. Environmental concerns for the marine environment, trends in legislation and the requirement for biological effects measurements

There is general growing concern over the fate and effect of chemicals in the marine environment. Around the world, concerns for the marine environment are focused on coastal waters (McIntyre, 1995) and the environmental challenges arising from pollution, and from urban and industrial development. Around 70% of marine pollution comes from land-based sources and their impact is concentrated in coastal areas (Holdgate, 1994). The principal sources of pollution occur from inputs of sewage, nutrients, agricultural run-off and synthetic organic compounds (Ducrotoy, 1994; Holdgate, 1994). The estuarine and other coastal marine environments have important roles in the disposal of effluents and wastes. The practices employed have operated on the principle that the receiving water has some capacity to tolerate disturbance due to anthropogenic inputs. This 'environmental capacity' of receiving waters for effluents and emissions is considered sufficient to allow their use to assimilate effluents and wastes without unacceptable harm (Pravdic, 1985). This concept, although accepted widely by regulators for managing the release of effluents and wastes into the marine environment, has not been deemed successful in preventing pollution (Jackson & Taylor,

1992). Consequently, the occurrence of unacceptable changes in the environment due to pollution has led a number of national and international bodies to accept the 'precautionary principle.' The 'precautionary principle' is a political principle that urges greater caution in the anticipatory phase of pollution control and focuses on the need for more preventative action, and the introduction of control measures not requiring proof of causality between contaminants and their effects (Stebbing, 1992). The 'precautionary principle' is now enshrined in environmental legislation and regulatory efforts to improve water quality have focused on the control of inputs of potentially toxic chemicals. The United Kingdom is signatory to the Convention for the prevention of Marine Pollution from Land-Based Sources [the Paris Convention now merged with the Oslo Convention to form the Oslo and Paris Commission (OSPARCOM)]. The United Kingdom is also a signatory to the Declarations of the North Sea Conferences. The United Kingdom is applying these measures to its inputs into all British coastal waters, not just the North Sea, and has been monitoring the achievement of these targets and attempting to deliver them via changes to discharge consents (Environment Agency, 1996a). A report on the quality status of the North Sea (OSPARCOM, 1993) concluded that, in most situations, pollution effects would be the result of multiple exposures to a variety of contaminants, and that in such circumstances the best measure of the integrated impact is through biological effects studies.

Regulatory authorities have acknowledged the utility of toxicity testing as part of a water quality-based approach for controlling toxic discharges and, in the United States of America, acute and chronic toxicity tests are key components of an integrated scheme involving the use of chemical specific and biological monitoring (US EPA, 1991a). Such biological tests are particularly important in the case of complex effluents, where the range of chemical components may be too great to allow accurate predictions of effect from chemical analyses alone. The utility of toxicity as a water quality-based control parameter for regulating

discharges depends on the relationship between measured toxicity in the receiving water and biological impact on the resident biota. The toxicity limit, if developed correctly from effluent toxicity tests, should protect aquatic biota if the discharged effluent meets these limits. The United States Environmental Protection Agency (US EPA, 1991a) provided data from several studies as evidence that effluent toxicity tests (chronic tests on ambient water samples) predict effects to resident aquatic communities and they concluded that "the results (of these studies), when linked together, clearly show that if toxicity is present after considering dilution, impact will also be present." The data from these studies were examined critically by Parkhurst (1995) who concluded that single species toxicity tests on ambient water samples have valid utility for indicating effects to aquatic communities provided that biological, toxicological and ecological variability are considered properly. The United States Environmental Protection Agency (US EPA, 1991a) also examined the prediction between toxicity test data on effluents and marine receiving water toxicity. Results from the study sites (79 samples) indicated a 94% accuracy when using marine and estuarine toxicity tests to predict receiving water impacts. Increasing recognition that toxicity tests, if applied correctly, can provide a valid means of regulating the discharge of chemicals has led to a wider application of this approach throughout Europe (Pedersen *et al.*, 1994).

In the United Kingdom, current regulatory practice relies heavily on the application of environmental quality standards (EQSs) to protect aquatic life, whereby, discharge consents are determined for a range of specific substances and limits are set to ensure that the EQS is not exceeded in the receiving water. Information on environmental fate and behaviour, and laboratory data on acute and chronic toxicity, are used to set safe concentrations which aim to avoid toxic effects in the environment. For many chemicals, this information does not exist and this uncertainty of effect is taken into consideration by the application of an arbitrary safety factor. A further limitation of the EQS approach is that potential interactions between

different chemicals in either the effluent or the receiving water are not accounted for. To date, the application of toxicity tests for the control and monitoring of discharges in the United Kingdom has been limited (Haig *et al.*, 1989; MacKay *et al.*, 1989; Hunt *et al.*, 1992; Johnson *et al.*, 1993). More recently, a national strategy for the implementation of toxicity-based consents has been proposed (Wharfe & Tinsley, 1995) and progress towards the implementation of this scheme has been made (Environment Agency, 1996b). The initial selection of toxicity test methods focuses primarily on measurement of acute toxicity, however, the intention to proceed with measurements of chronic toxicity is stated (Environment Agency, 1996b).

Measurement of toxicity in the receiving water (ambient tests) can be used to determine if toxicity is a contributory cause to effects on resident biological communities. Most surface waters do not show acute toxicity, so to determine if toxicity occurs in water samples, chronic toxicity must be measured (Mount, 1988). In the United Kingdom, the need to provide measurements of effects in the receiving water has been identified (Wharfe & Tinsley, 1995) and this implies the need for bioassay techniques which are sufficiently sensitive to detect chronic as well as acute effects.

1.2. Current approaches to the toxicity assessment of effluent discharges and potential limitations

Regulatory approaches to ecotoxicology utilise toxicity tests on individual organisms that can be carried out quickly, easily and, hence, inexpensively to make predictions about the potential long-term impact of toxicants at an ecological level (Maltby & Calow, 1989). The ability of data derived from single-species laboratory toxicity tests to reliably predict effects on higher levels of biological organisation has been widely debated (Cairns & Pratt, 1993; Forbes & Forbes, 1994). Extrapolation from any artificial system, including those used for toxicity

tests, to a complex natural system is a challenging and difficult task (Cairns & Pratt, 1989). For example, contaminants in estuarine and coastal waters usually exist as complex mixtures, often at low concentrations (Matthiessen *et al.*, 1993). In these situations, where ambient toxicity may be low or marginal, confounding factors such as biological variability, community resilience, habitat, other sources of toxicity and the variability of single species tests may obscure or overwhelm any effects toxicity may have on the resident community (Parkhurst, 1995). Aquatic communities are too complex and too poorly understood to discern if toxicity is causing impairment with any practical amount of effort (Mount, 1988). Under these circumstances, and for the foreseeable future, single-species laboratory tests are likely to continue as the most widely used method for evaluating the potential hazards of chemicals (Forbes & Forbes, 1994).

Acute toxicity tests are used widely to assess the ecotoxicological effects of effluent discharges but the threshold for chronic effects is generally a more crucial value than the median lethal threshold (Birge & Black, 1985). Chronic effects of contaminants are often inferred or estimated from observations made during acute toxicity studies but there is considerable doubt whether acute toxicity can be used to predict chronic effects with a useful degree of certainty (Forbes & Forbes, 1994). Ernst (1980) concluded that, for highly persistent substances having low water solubility and high bioconcentration potentials (e.g. many organic chemicals), the median lethal concentration can provide only marginal information. However, the ability to conduct chronic toxicity investigations is frequently limited due to inadequate knowledge of the culturing requirements of the test species and other biological factors (Abel, 1991). Limitations are often imposed in regulatory ecotoxicology where, for example, there is often a need to conduct chronic toxicity tests on marine organisms outside their normal breeding season or beyond their normal geographic range. In addition to

these factors, conducting life-cycle tests on many marine species is relatively time consuming and expensive.

Attempts to provide more rapid and cost-effective alternatives to established chronic toxicity tests have focused on the development of test methods using sensitive early life-history stages (McKim, 1977, 1985; Mount & Norberg, 1984; Norberg & Mount, 1985). These short-term sublethal tests (often referred to as chronic estimators) generally provide an improved prediction of chronic effects compared with acute toxicity tests (Norberg-King, 1989). The United States Environmental Protection Agency has developed a suite of short-term sublethal tests for freshwater and estuarine/marine environments utilising species of microalgae, macroalgae, invertebrates and fish (US EPA, 1991bc). Typically, tests are seven days or less in duration and attempt to estimate chronic toxicity by measuring sublethal effects such as development, growth and reproduction. The State of California has extended this approach to develop sublethal test methods for species (e.g. abalone, giant kelp, mysid shrimp) indigenous to the Pacific coastal waters of the west coast of the United States of America (Anderson & Hunt, 1988; Hunt & Anderson, 1989; Martin *et al.*, 1989). Concerns have been expressed that relatively short exposure durations, even for sensitive life stages, may not reflect accurately biological effects due to prolonged exposure to toxicants (Suter, 1990). Consequently, while short-term sublethal tests for estimating chronic toxicity may have practical and economic value, they should be validated against chronic exposures. According to Suter (1990), "accurate predictions of chronic toxicity still require chronic tests."

1.3. Research needs

The evolution of biomonitoring requirements in Europe and in the United Kingdom is expected to follow a similar approach to that used by the United States Environmental Protection Agency and, if so, the priority for biological methods development will focus on

procedures that can measure both acute and chronic toxicity. Generally, the development of estuarine and marine chronic toxicity test procedures in the United Kingdom has not kept pace with research initiatives to develop freshwater chronic toxicity test methods (European Commission, 1995). The oyster embryo and algal growth bioassays have been used on research cruises to monitor the biological water and sediment quality in the North Sea and English Channel (Thain, 1992a, 1992b; MAFF, 1994), and these methods are amongst those under consideration for the toxicity assessment of effluents in the United Kingdom (Wharfe & Tinsley, 1995; Environment Agency, 1996b). Although reached rapidly (24 h), the end point of the oyster embryo test (normal development of the embryo to the D-larval stage) includes both lethal and sublethal components, and is, consequently, very sensitive to many contaminants (Thain, 1991). Although the oyster embryo and algal growth tests provide sublethal responses, the relatively short exposure durations (24 and 72 h respectively) may not accurately reflect any effect resulting from prolonged chronic exposure to toxicants.

1.4. Criteria for the selection of chronic toxicity test methods

Based on existing information concerning the toxicity assessment of effluents, the development of chronic toxicity methods will require a number of important criteria. The key points are discussed below and more detailed information concerning the criteria for selection of test species and the objectives of bioassays are published (Cairns & Pratt, 1989; Richardson & Martin, 1994).

1.4.1. Use of sensitive life stages

The sensitivity of the larval stages of fish and invertebrates to a range of single toxicants has been reported widely (McKim, 1977, 1985; Patin, 1982). Tests, therefore, should incorporate sensitive early life-history stages of indigenous species as their use may

better indicate the potential impact of discharged wastes on marine populations (Martin & Richardson, 1995).

1.4.2. Use of realistic and relevant end points

To translate individual organism responses to higher levels of organisation, Chapman (1995) urged the development of tests, and biological end points, that are more ecologically relevant. Functional responses based on reproduction appear to provide one of the most sensitive and ecologically appropriate measures of stress resulting from exposure to toxic substances (Evans & McNaught, 1988). Impairment of reproduction is recognised as a key parameter for conducting hazard assessment of the effect of xenobiotics at the concentrations encountered in the natural environment (Neilson *et al.*, 1994). Chapman (1995) advocated the use of life-table data experiments in which cohorts of an appropriate test organism are exposed to a full life cycle with end points including survival (longevity), growth (rate and type of development) and reproductive success (fecundity). These end points are used to compute the intrinsic rate of natural increase, which has ecological relevance but has rarely been used in toxicity testing (Bechmann, 1994).

1.4.3. Laboratory culture and the availability of test organisms

Practical considerations include ease of handling and the ability to culture the organism in the laboratory. One of the major constraints of bioassays that depend on early developmental stages is that their availability is likely to be seasonal (Stebbing & Harris, 1988). Ideally, test organisms should be available throughout the year, however, this may be difficult to achieve for some species.

1.4.4. Establishing the links between contaminant effects at different levels of biological organisation

A recurring theme in the ecotoxicological literature is the need for greater insight and understanding into the links between pollutant effects across different levels of biological organisation (Cairns & Pratt, 1989; Maltby & Calow, 1989). Given the inherent difficulty in trying to extrapolate from single-species tests on individuals to populations and communities of organisms, it has been suggested that ecotoxicological research should focus directly at the level of the population and above (Forbes & Forbes, 1994). The need to translate individual organism responses to higher levels of organisation has been identified already but few attempts have been made to demonstrate a causal relationship between effects at different levels of biological organisation.

Life-table data analysis, and subsequent computation of the population parameter known as the intrinsic rate of natural increase, is derived from observations made on individual organisms. Measurement of individual organisms, however, does not take into account population effects that occur through interactions between individuals (competition and food availability). These latter factors are likely to be most important in populations influenced by density-dependent factors. The development of test methods based on the measurement of individual organisms and populations of the same species would improve our understanding of how contaminants impact on populations rather than individuals. Comparisons between the individual organism and population measurements would also identify the utility of individual measurements for predicting effects at a higher level of biological organisation.

1.5. Copepods as candidate species for toxicity tests

A recent review of priorities for revision and development of Organisation for Economic Co-operation and Development (OECD) Test Guidelines in aquatic toxicity testing

(OECD, 1995) assigned highest priority to the development of acute and chronic tests with species of marine Crustacea. Sublethal and chronic laboratory toxicity test procedures have been developed for both calanoid and harpacticoid copepods, and the biological end points for such tests include survival and development of the early life-history stages and aspects of reproduction.

1.5.1. Calanoid copepods

Calanoid copepods have, because of their ecological importance in estuarine and marine food webs (Bushong *et al.*, 1990), been used widely to determine the effect of pollutants. The development of calanoid copepod toxicity test procedures has focused on species from two genera, *Eurytemora* and *Acartia*. Test methods for *Eurytemora* describe procedures to determine the effect of chemicals on survival and development from the naupliar stage to reproductive maturity (Hall *et al.*, 1988; Hall *et al.*, 1995), on egg production by individual females (Berdugo *et al.*, 1977) and on the numbers of offspring produced by adult populations (Marcy, 1986). Test methods for *Acartia* describe effects on survival and development of the naupliar stages (Ward *et al.*, 1979; Bushong *et al.*, 1990), egg production by groups of adult females (Johansen & Mohlenberg, 1987), egg production and viability (hatching) by individual or groups of adult females (Tester & Costlow, 1981; Sunda *et al.*, 1987) and increases in population number (Girling, 1989). Procedures to measure effects on egg production and hatching success have been described also for species of *Centropages* (Cowles & Remillard, 1983; Capuzzo, 1985), *Temora* and *Pseudocalanus* (Capuzzo, 1985).

Of these studies, four (e.g. Berdugo *et al.*, 1977; Ward *et al.*, 1979; Hall, 1988; Girling, 1989) described exposure durations ranging from 13 to ≈30 days, the remainder (8/12) described exposure periods of 4 - 8 days. It could be argued, therefore, that the duration of the majority of the work carried out to date may not reflect accurately the potential long-term

effect of contaminant exposure to these organisms. Furthermore, the studies focused typically on only one aspect of the copepod life cycle (e.g. egg production), ignoring the potential effects of a contaminant on other components of the life history. The development of robust chronic toxicity test procedures for calanoid copepods is not without problems and these difficulties have limited the application of such tests in regulatory ecotoxicology. Laboratory culture of many calanoid species is difficult and labour intensive (Davis, 1983, Marcy, 1986), and the nutritional requirements of many species are best met by diverse (e.g. mixed algal species) diets (Kleppel & Burkart, 1995). Calanoids, particularly the adult stages, may be sensitive to handling and, in toxicity tests, this may translate into levels of control mortalities that are unacceptable for compliance with regulatory protocols (Marcy, 1986). Measures of egg production are subject to several factors that may lead to erroneous conclusions of the data. These factors include cannibalism of eggs by some species of calanoids (e.g. *Calanus*), particularly in small test volumes (Runge, 1984); eggs failing to hatch because of infertility (Ianora & Poulet, 1993) or poor nutritional quality of the food (Jonasdottir & Kiorboe, 1996) and high rates of egg mortality due to density-dependant factors such as disease (Peterson & Kimmerer, 1994). Many of the smaller copepod species that inhabit shallow coastal waters (e.g. *Acartia* spp, *Temora* spp and *Centropages* spp) have strategies adapted to shallow-water communities, producing resting (diapause) eggs which overwinter in the sediment to be released the following year (Williams *et al.*, 1994). The production of diapause eggs in response to crowding has been reported for *Eurytemora affinis* (Ban, 1992; Ban & Minoda, 1994). These aspects of the reproductive biology of calanoids present a serious challenge to the development of scientifically robust and practicable chronic toxicity test procedures.

1.5.2. Harpacticoid copepods

Epibenthic and phytal-dwelling harpacticoid copepods possess a number of attributes that make them particularly useful organisms for toxicity testing. For example, members of the genus *Tisbe* possess naupliar larvae which, after embryonic development, hatch from the egg sac directly onto the substratum inhabited by the adults (Hicks & Coull, 1983). *Tisbe* spp demonstrate high rates of juvenile survival and development, possess relatively short generation times and sustain high levels of fecundity (Bergmans, 1981, 1984). Multiple broods of offspring can be fertilised from a single copulation, therefore, females can be raised individually to provide precise measurements of longevity and reproductive parameters (e.g. number of broods, brood size). These biological attributes facilitate the measurement of development, reproduction and life-table analysis, which provide sensitive and ecologically relevant end points that can be used to determine the potential chronic toxicity of contaminants.

Coull and Chandler (1992) reviewed over 200 field, laboratory and mesocosm studies concerning meiofauna and pollution. Of 68 laboratory *in vitro* tests, approximately 39 (57%) were conducted with harpacticoid copepods and 96% of these used species from only three genera [*Tisbe* (40%), *Tigriopus* (29%) and *Nitocra* (28%)]. Both *Tisbe* and *Tigriopus* are non infaunal epibenthic or phytal copepods; *Nitocra* occupies a similar niche although some species burrow in sediments (Coull and Chandler, 1992). These genera have been used predominantly to determine the acute toxicity of contaminants in the aqueous phase, however, in nature many contaminants are associated with sediments. Concerns over the fate and effect of contaminants in sediments has led to the recent development of acute sediment toxicity test methods, again, using infaunal harpacticoids (Green *et al.*, 1993).

Relatively few studies have reported the measurement of sublethal and chronic effects of contaminants to harpacticoids. Ustach (1979) used *Nitocra affinis* to study effects on brood

size of the water soluble fraction of crude oil, and Bengtsson and Bergstrom (1987) described a flow-through fecundity test that measured the total number of offspring produced by *Nitocra spinipes* after 13 days exposure. Techniques were described for measuring the effect of toxicants on naupliar survival and development (Le Dean & Devineau, 1987) and offspring production (Lassus *et al.*, 1984) by *Tigriopus brevicornis*. Dalla Venezia *et al.* (1980) examined the effects of a polychlorinated biphenyl (PCB) on the survival of newly hatched nauplii to maturity and female fecundity in *Tisbe bulbisetosa*. Verriopoulos and Hardouvelis (1988) isolated individual female *Tisbe holothuriae* and determined the sublethal effects of zinc on the survival and fertility of four successive generations. Changes in the population size of *T. holothuriae*, following long-term exposure (24 days up to 30 weeks) to toxicants, were described by Hoppenheit (1977), Hoppenheit and Sperling (1977) and, more recently, by Brand *et al.* (1986). Bechmann (1994) described the use of life tables, in which nauplii were exposed from hatching to day at death, to evaluate the effect of toxicants to *Tisbe furcata*. Sublethal and chronic sediment toxicity test methods have been developed also for measuring the effect of sediment-bound contaminants to infaunal harpacticoids (Chandler & Scott, 1991; Di Pinto *et al.*, 1993; Green & Chandler, 1996).

The development of test methods to study the effect of contaminants on harpacticoids in the laboratory may provide greater opportunity to establish cause and effect between contaminants and biological impact on the meiofauna in the field. A review of the use of meiobenthos (including harpacticoid copepods) in studies of marine pollution (Rees *et al.*, 1991) highlighted the difficulties in distinguishing pollution-induced from natural changes and concluded that knowledge of the ecotoxicology of the meiobenthos is still very poor and that much more work remains to be done.

1.6. Initial stages in the consideration of *Tisbe battagliai* as a candidate test organism

The ability to conduct life-table analysis and population experiments were considered important criteria for the development of chronic toxicity test methods. Based on the selection criteria discussed above, harpacticoid copepods appeared to offer potential for the development of such test methods and a review of the literature highlighted members of the genus *Tisbe* as candidate test organisms. Selection of a suitable test organism began when a single egg sac female of an unidentified species of harpacticoid copepod was isolated from a flowing seawater tank at the Brixham Environmental Laboratory (1987). Offspring from this female were used to initiate a continuous laboratory culture and specimens from this culture were identified subsequently as *Tisbe battagliai* (Fig. 1.1). The development of toxicity test methods using *T. battagliai* proceeded via a tiered approach consisting of acute, short-term sublethal and chronic procedures. The development of acute and short-term sublethal methods, although integral to the development of chronic methods reported in this thesis, are described fully elsewhere (Hutchinson & Williams, 1989; Williams, 1992; Williams *et al.*, 1993; Hutchinson *et al.*, 1994), however, a brief summary of these experiments is provided below.

Acute toxicity test methods provide an option of 48 h static or 96 h semi-static exposures using the naupliar, copepodid or adult female life-history stage (Williams & Thompson, 1992a, 1992b). Naupliar and copepodid stages are generally more sensitive than the adult female but the naupliar stage, because of its small size (< 100 μm in length at 24 h), is more difficult to handle than the copepodid stage. Therefore, for practical purposes, the copepodid stage is the preferred choice of life stage for acute toxicity tests and this method has been included in an international standard method (ISO, 1995). The acute test methods were evaluated as part of the Paris Commission (PARCOM) interlaboratory ring test involving a

range of species selected from the algae, herbivore and sediment reworker groups and the results have been published (PARCOM, 1992; Bjornestad *et al.*, 1993).

Test methods have been developed also for estimating the chronic toxicity of single chemicals and industrial effluents. These procedures utilise two different life-history stages and measure effects on adult reproduction, and naupliar survival and development, in exposure periods lasting up to 10 days (Hutchinson & Williams, 1989). Individual females are isolated from time of first egg sac production and the survival and number of offspring per female monitored for periods of 6 - 10 days, sufficient time for each female to produce 2 - 3 broods. Also, the survival and development of < 24 h old nauplii are monitored for periods of 7 - 10 days, sufficient time for the nauplii to develop into the late copepodid (C4-C5) or adult stage at 20°C (Williams & Jones, 1994). Short-term sublethal methods were further evaluated using metal toxicants (Hutchinson *et al.*, 1994). The acute to chronic ratio [lowest 96 hour LC50 to 8 day no observed effect concentration (NOEC) for sublethal tests with nauplii and adult females] was approximately 18 for cadmium, 5 for hexavalent chromium and 11 for copper, indicating that significant biological effects may occur at toxicant concentrations substantially lower than those causing acute lethality. A biomonitoring programme using acute and short-term sublethal tests was undertaken to provide a broad base of acute and estimated chronic values for important industrial effluents (Williams *et al.*, 1993). The sensitivity of *Tisbe battagliai* is similar to that reported for other species of marine crustaceans (PARCOM, 1992; Bjornestad *et al.*, 1993; Hutchinson *et al.*, 1994) yet is sufficiently robust for the culture and test methods to be used in shipboard experiments. Measurements of survival, growth and development from the naupliar to the copepodid stage after 96 h were employed successfully in shipboard experiments on samples of surface microlayer, water column and sediment elutriates from a contamination gradient in the German Bight of the southern North Sea (Williams, 1992). These short-term sublethal tests provided an estimate of potential chronic effects,

however, to be of most value these end points should be validated with results from chronic exposures.

1.7. Research objectives of the thesis

1.7.1. To develop chronic toxicity test methods using *Tisbe battagliai*

The primary objective of the research programme was to develop chronic toxicity test procedures using the marine harpacticoid *Tisbe battagliai*. The development of test methods provided a number of options including measurements of naupliar development, adult reproduction and complete life-cycle studies in which the period of exposure included all the life-history stages. These methods involved different exposure periods hence there are implications for the time and cost of performing such studies, constraints often imposed by the development of regulatory test methods.

1.7.2. To establish information on the basic biology of *Tisbe battagliai*

Information on the biology and life history of *Tisbe battagliai* is very limited and only one other study provided demographic data for this species (Bergmans & Janssens, 1988). Inadequate knowledge of the basic biology of test organisms remains an obstacle to the development of toxicity test methods. Such knowledge is critical for the development of chronic test methods where factors such as basic nutritional requirements can have a significant influence on test end points such as growth and reproduction. To facilitate this objective, the development of chronic toxicity test methods based on development, reproduction and life-table analysis proceeded alongside investigations of the influence of key environmental variables (temperature and food availability) on the biology of *T. battagliai*. Results from these studies established the optimal conditions for both culture and testing of this species in

the laboratory. There is considerable interest in the life history and demography of meiofaunal organisms and quantitative studies, particularly the aspects of reproduction in harpacticoid copepods, promises new insights, perhaps even of broad ecological significance (Bergmans, 1984a).

1.7.3. To improve the environmental applicability of laboratory toxicity tests

Chronic toxicity tests are performed frequently under test conditions that, for specific organisms, are close to optimal. These conditions may not be representative of those in natural systems where organisms respond to the dynamic conditions of their environment (e.g. resource availability). As an alternative, studying the combined effect of these environmental parameters under laboratory conditions may significantly improve our understanding of how key environmental variables affect organisms and their response to toxicants under more environmentally realistic exposure situations.

1.7.4. To develop test procedures for linking effects at different levels of biological organisation

The need to translate individual organism responses to higher levels of biological organisation has been discussed previously (Section 1.4.4). An additional objective of this aspect of the research was to provide an experimental approach for investigating the potential links between effects at the individual and population level within the same species. To further this objective, chronic toxicity test methods based on the measurement of individuals were supplemented with measurements of growth in populations of *Tisbe battagliai*.

1.8. An outline of the thesis

Chapters 2 to 4 describe the development of the chronic toxicity test methods for measuring effects on postembryonic development (Chapter 2), reproduction (Chapter 3) and the complete life cycle (Chapter 4) of *Tisbe battagliai*. These experiments were conducted at different temperatures (15, 20 and 25°C) and food concentrations (0, 83, 208, 520, 1300 and 3250 μgC l^{-1} of a unialgal diet of the alga *Isochrysis galbana*) to evaluate the potential interaction between these key environmental factors and their influence on the biology of *T. battagliai*.

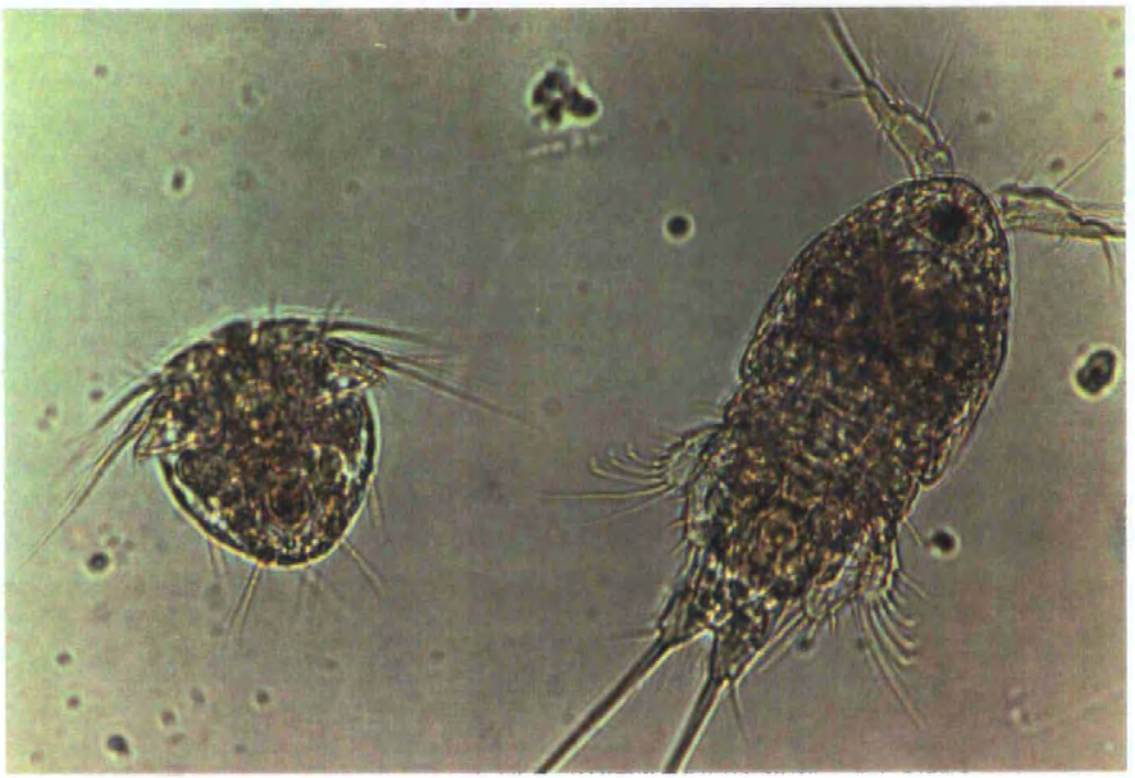
Chapters 2 to 4 established the relative importance of food quantity on the population dynamics of *Tisbe battagliai* but questions concerning food quality, and the ability of a unialgal diet to provide an optimal source of nutrition, remain unresolved. In Chapter 5, the effect of food quality on postembryonic development, reproduction and the complete life cycle (life-table analysis) is described. Experiments were conducted at a single temperature (20°C) and food concentration (3250 μgC l^{-1}), and comparisons were made between unialgal and mixed species algal diets consisting of *Isochrysis galbana* and *Rhodomonas reticulata*.

In Chapter 6, the chronic toxicity test methods were evaluated further using pentachlorophenol (PCP) as a reference toxicant. The objective was not simply to generate traditional chronic toxicity information, where measurements are made at prescribed (near optimal) conditions of temperature and food, but to investigate the potential interaction between toxicant (PCP), temperature and food availability (food quantity and quality) and their effects on the population dynamics of *Tisbe battagliai*. The variables (temperature, food and toxicant) used in these experiments imposed a number of constraints on the experimental design and preliminary toxicity experiments were required before definitive experiments could proceed. Based on preliminary results, the experimental design for definitive postembryonic and reproduction experiments consisted of three temperatures (15, 20 and 25°C), three food

concentrations (1300, 2055 and 3250 µgC l^{-1} of *Isochrysis galbana*) and four concentrations of PCP (0, 10, 32 and 100 µg l^{-1}). A different experimental design was adopted for the complete life-cycle experiments (life-table analysis) with greater emphasis on the potential effects of food quality on population dynamics. Life-table experiments proceeded at a single temperature (20°C) and *Tisbe* were exposed to PCP concentrations of 0, 10, 32 and 100 µgC l^{-1} and two food concentrations (1300 and 3250 µgC l^{-1}), consisting of single (*I. galbana*) and mixed species (*I. galbana and Rhodomonas reticulata*) algal diets.

The test methods described in Chapters 2 to 6 employed measurements from individuals, however, this approach to population dynamics does not take into account population effects that occur through competitive interactions between individuals. In Chapter 7, test methods for measuring the potential effect of contaminants on populations of *Tisbe battagliai* are described. As with previous experiments, the potential interaction between environmental variables and organism response to toxicants was the main theme of the investigation. In Chapter 7, however, the objective was to examine the effect of food quantity and quality on the response of *T. battagliai* populations to prolonged exposure (11 weeks) to sublethal concentrations of a toxicant. Experiments were performed at 20°C and the experimental design consisted of two exposure concentrations (0 and 100 µg l^{-1} PCP) and three food concentrations (520, 1300 and 3250 µgC l^{-1}), each consisting of single (*I. galbana*) and mixed species (*I. galbana and Rhodomonas reticulata*) algal diets.

Chapter 8 provides a general discussion of the key findings from the research programme and these are discussed within the framework of the research objectives set out at the start of this study (Section 1.7). Where appropriate, recommendations are made for further research.



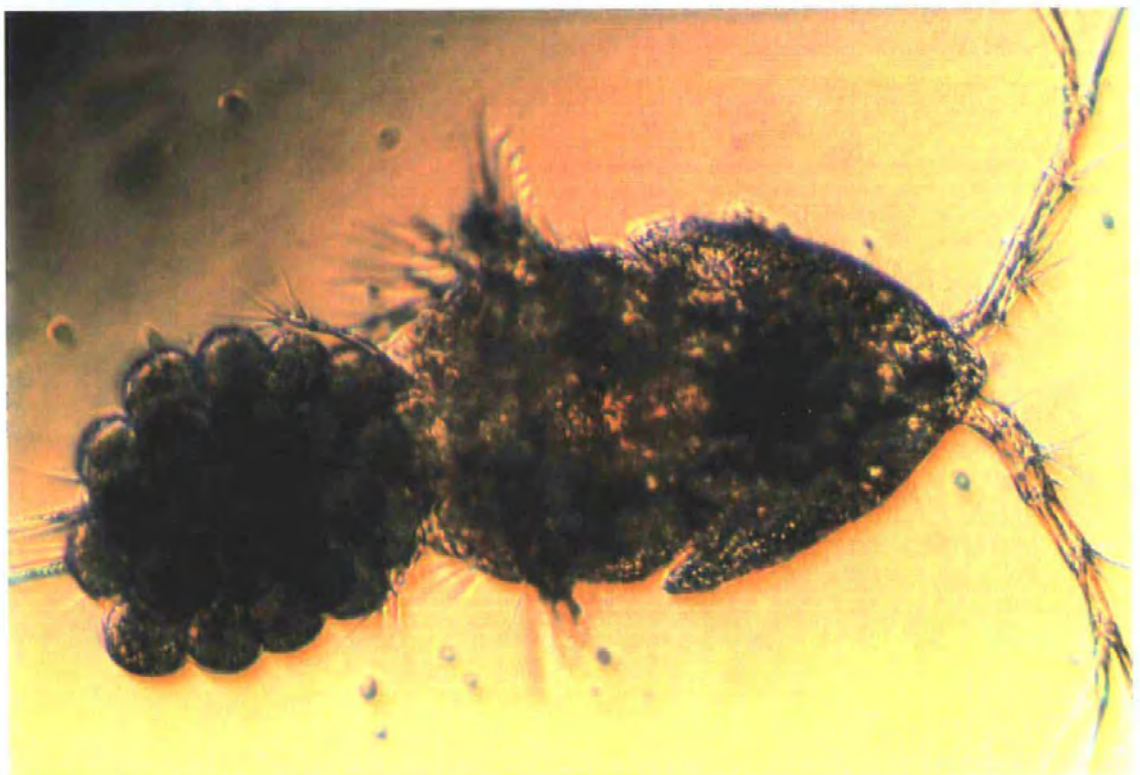


Figure 1.1. The naupliar (upper left), copepodid (upper right) and adult female life history stages of *Tisbe battagliai*.

CHAPTER 2

EFFECTS OF TEMPERATURE AND FOOD QUANTITY ON POSTEMBRYONIC DEVELOPMENT OF *TISBE BATTAGLIAI* (COPEPODA: HARPACTICOIDA)

This Chapter was presented as a talk at a symposium on the Developmental Biology of Marine Organisms, organised by the Marine Biological Association and the Society for Experimental Biology, Plymouth, April 19-21, 1993. It is now published as a paper in the *Journal of Experimental Marine Biology and Ecology* 183, 283-298 (1994).

2.1. INTRODUCTION

Harpacticoid copepods form a major component of the marine meiobenthos and constitute an important food resource for larval and juvenile fish (Hicks & Coull, 1983; Gee, 1989). Members of the marine meiobenthos have been used to determine the effects of anthropogenic perturbation of aquatic systems (Coull & Chandler, 1992) and the development of laboratory culture techniques for a number of harpacticoid species has allowed their use in toxicity testing (Lassus *et al.*, 1984; Bengtsson & Bergstrom, 1987; Hutchinson & Williams, 1989; Chandler & Scott, 1991; Strawbridge *et al.*, 1992). Few studies, however, have detailed the duration of the various harpacticoid life-history stages, and information on the influence of environmental factors on harpacticoid development and reproduction is sparse. The aim of this chapter was to establish the combined effect of temperature and food concentration on the postembryonic development of the marine harpacticoid *Tisbe battagliai* Volkmann-Rocco, as a prelude to assessing its potential use as a marine bioassay organism. Marine benthic copepods of the genus *Tisbe* (Copepoda: Harpacticoida) are particularly suitable laboratory organisms in this context, as they have a wide geographical distribution, possess short life cycles, require minimal space and equipment for testing, are comparatively easy to culture in the laboratory (Battagliai, 1970; Williams, 1992), and animals at all developmental stages can be harvested from cultures at any time of the year. In the field, members of the genus *Tisbe* nearly always occur as multispecies guilds, and these guilds often include several members of a single group of sibling species (Bergmans & Janssens, 1988). Thus, the taxonomy of the genus is complicated by the existence of sibling species, which are identified by detailed investigations of morphology and cross-breeding experiments (Volkmann-Rocco, 1972; Volkmann-Rocco & Battagliai, 1972). Details on the morphology and phylogeny of the naupliar and copepodid stages of the Harpacticoida are available (Dahms, 1990, 1991, 1992, 1993; Dahms & Schminke, 1995), however, the postembryonic developmental stages of only a few species of

Tisbe have been described (Dahms & Bergmans, 1988). *Tisbe battagliai*, a sibling species of the *holothuriae* group, has been found in shallow waters in coastal regions of Europe and the Atlantic coast of the USA (Volkmann-Rocco, 1972). Whilst its sibling species, *T. holothuriae* Humes, is a popular experimental animal (Fava & Crotti, 1979; Brand, 1985; Miliou & Moraitou-Apostolopoulou, 1991abc; Miliou, 1992), information on the biology of *T. battagliai* is limited to laboratory studies of food preference (Vanden Berghe & Bergmans, 1981), and the influence of salinity on the life history of *T. battagliai* and *T. holothuriae* in pure and mixed cultures (Bergmans & Janssens, 1988).

2.2. MATERIALS AND METHODS

2.2.1. Copepods

A population of *Tisbe battagliai* was established (May 1987) at the Brixham Environmental Laboratory from nauplii collected from a single adult female isolated from a flowing seawater tank. Experimental cultures of *T. battagliai* were maintained in filtered (0.2 μm), natural seawater (approximately 35‰) obtained from Tor Bay (Devon), and held in constant temperature rooms at 15, 20 and 25°C and a photoperiod of 16 h light: 8 h dark. Culture vessels were rectangular polystyrene tanks (25 cm long, 15.5 cm wide & 15 cm tall) containing 3 l of seawater which was replaced once per week with freshly filtered seawater which had previously been aged for 7 days prior to filtration. Copepods were fed with the prymnesiophyte *Isochrysis galbana* Parke, grown in a continuous culture chemostat (see next section). Cultures were fed initially 5 ml l^{-1} (equivalent to 2.5×10^5 cells ml^{-1} in 3 l) of an algal stock suspension containing 5×10^7 cells ml^{-1}, at least once per week (when the seawater was renewed). Cultures were observed daily and a similar quantity of algae was added provided the animals had cleared the previous food suspension from the water.

2.2.2. Algal culture

Isochrysis galbana (Strain CCAP 927/1, Culture Centre of Algae and Protozoa) was grown in a chemostat culture using the growth medium described in Table 2.1. Filtered (0.2 μm) seawater was sterilised in an autoclave and stock solutions were added (1.0 ml l^{-1} solution A and 0.05 ml l^{-1} solution B) via aseptic transfer with a 0.2 μm filter. Sterile medium was held in 10 l glass reservoirs and pumped via a multichannel peristaltic pump into glass reaction vessels holding 3 l of culture volume. The reaction vessels were located in a constant temperature room (20°C) and illuminated with four white fluorescent bulbs on a photoperiod of 16h light: 8h dark. Cultures were stirred by constant, vigorous aeration with 0.2 μm-filtered room air. Cell density in the culture was maintained at approximately 1 x 10^7 cells ml^{-1} by a 30% daily replacement of the culture volume. Culture outflow was pumped from the reaction vessels and the cells collected daily for preparation of food suspensions. The harvested algae were centrifuged (5 K for 15 mins) and, after discarding the supernatant, the algal cells were resuspended in filtered (0.2 μm) seawater to provide a stock suspension containing 5 x 10^7 cells ml^{-1}.

2.2.3. Test procedure

Postembryonic development of most harpacticoid copepods includes six naupliar stages and six copepodid (or copepodite) stages; the sixth copepodid stage corresponds to the mature adult and is reached after 11 moults (Hicks & Coull, 1983). Copepods undergoing this full developmental sequence, therefore, pass through 11 juvenile stages (N1-N6, C1-C5). Copepod development rates are a potentially useful indicator of response to environmental variables and can be measured as the time taken to reach the individual moult stages. In this experiment, the progress of individual nauplii was followed from hatching through to adulthood, however, due to the rapid development time of the naupliar stages, the total development time to complete the six naupliar

stages (time between hatching and moult into C1) was used as the criterion of naupliar response. For the copepodid stages, moulting was less frequent and individual stage durations were measured.

Experiments were carried out in temperature controlled rooms (± 1°C) with a photoperiod of 16 h light: 8 h dark. Newly-released nauplii (< 5 h old) were reared to adult at three different temperatures (15, 20 and 25°C) and six concentrations of *Isochrysis galbana* (0 - 3250 µgC l^{-1}). Cohort synchrony was achieved using nauplii released within 5 h from parturient females (n = ≈ 50) isolated from the main cultures in a crystallising dish containing 200 ml of seawater and ≈ 2 x 10^5 cells ml^{-1} *I. galbana*. To limit the transfer of algal cells, nauplii were sieved carefully through a 62 µm nylon mesh and resuspended in freshly filtered seawater before being added to the experimental containers. The latter were multi-well, disposable, polystyrene tissue culture plates with close-fitting lids. Stock suspensions of *I. galbana* were diluted with filtered (0.2 µm) seawater to provide concentrations of 0 (no algae added), 83, 208, 520, 1300 and 3250 µgC l^{-1}; each algal concentration increasing stepwise by a factor of 2.5. Corresponding values (mean measured), based on algal cell numbers, ranged from approximately 0.12 - 4.6 x 10^5 cells ml^{-1} (83 - 3250 µgC l^{-1}). Carbon values and cell concentrations for the freshly prepared algal stock suspensions were determined using a Dohrman DC-190 high temperature carbon analyser and a Model ZB Coulter Counter respectively. Algal stock suspensions were stored in a refrigerator in the dark and used within 7 days.

Ten nauplii (< 5 h old) were exposed to each combination of temperature and food concentration until the adult stage was reached. Individual nauplii were added randomly to 10 wells of tissue culture plates, each containing 2 ml of test solution. Due to their small size (80 - 100 µm in length) and mobility, it was difficult to view the nauplii in relatively large water volumes and a test volume of 2 ml was chosen to enable easy observation and counting. Nauplii were transferred from the culture dish to the test chambers using a finely-drawn out pasteur pipette. Transfers and observations during the experiments were carried out with a Wild M8 binocular stereomicroscope, using darkfield illumination. Observations for moulting and mortality were made at 24-hourly

intervals and the surviving copepods were transferred to duplicate test chambers containing freshly prepared test solutions. Temperature (daily), and the pH and dissolved oxygen concentration (at least weekly) was measured in each treatment.

The temperature range investigated in this study (15 - 25°C) corresponded to the average seasonal maxima in British and Mediterranean coastal waters, however, in strongly seasonal coastal waters there are times of the year (winter) when water temperatures decline to much lower values. For example, local water temperatures measured in Tor Bay, Devon between November 1992 and October 1993 ranged from approximately 5 - 16°C, and 10 year averages of monthly mean sea temperatures measured in Plymouth Sound, Devon between 1947 - 1956 ranged from 7.86 - 16.2°C (Cooper, 1958). Temperature data are also available from studies of the ecology of *Tisbe* spp inhabiting environments (in Italy) subject to wide fluctuations in physical factors. Recorded temperature values ranged from 6 - 29.7°C in the Lagoon of Grado (Fava, 1983), ≈ 5 - 25°C in the Lagoon of Venice (Fava & Volkmann, 1975) and 4 - 28°C in the Scardovari estuary (Fava & Volkmann, 1977).

2.2.4. Comments on the test procedures

Copepod development rates were followed successfully using the experimental procedures described here although certain aspects could have been improved. Naupliar development proceeded rapidly, especially at higher temperature, and daily observations were inadequate to define the stage durations for the individual naupliar stages. More frequent measurements (every 6 or 12 h) would be required to define naupliar durations at these temperatures and would also have provided more precise measurement of the copepodid durations. Despite the small number of copepods per treatment, variability in the data was low, but increased as food concentration became limiting at algal concentrations of 520 $\mu gC\ l^{-1}$ and lower. Due to the small sample size, development times for the individual sexes was not reported, however, Bergmans (1981a) showed

that male *Tisbe furcata* needed less time than females to complete development. Initial attempts to measure copepod size at adulthood were made, however, after sacrificing the copepods in 3% buffered formalin, flexing of the body (between the prosome and urosome), and arrangement of the body appendages prevented accurate measurement. Consequently, size determinations were not made, however, later attempts to measure copepod body size were facilitated by the use of propylene phenoxytol (2 - phenoxyethanol) as a relaxing agent. Although size measurements were not made, visual observation of the copepods as they reached the adult stage suggested smaller body size for copepods in food-limited treatments compared with those in high food concentrations (1300 and 3250 $\mu gC\ l^{-1}$). Further experiments are required to investigate any relationship between development rates (moulting) and body size in response to temperature and food concentration.

2.2.5. Statistical analysis

Statistically significant effects on copepod survival were calculated using Fisher's Exact Test, a contingency table procedure (Finney *et al.*, 1963). The data for copepod development times were tested for equality of variances prior to analysis of variance techniques (Sokal and Rohlf, 1995). The interaction of food concentration and temperature on copepod development times was tested using 2-way analysis of variance (ANOVA), using a 3 (temperature) x 4 (food concentration) factorial design. Food concentrations lower than 208 $\mu gC\ l^{-1}$ were excluded from these analyses due to high mortality. In the absence of any interaction, the independent effects of temperature and food concentration on development times were determined using 1-way ANOVA followed by *t*-tests (two-tailed) to identify significant differences between the treatment means.

2.3. RESULTS

2.3.1. Survival

Mortalities recorded during development from the nauplius to the adult stage are shown in Table 2.2. In treatments receiving no algal food (0 $\mu gC\ l^{-1}$), only 1 copepod survived beyond the naupliar stage and mortalities ranged from 80 - 100% over the temperature range 15 - 25°C. All copepods survived in algal concentrations of 1300 and 3250 $\mu gC\ l^{-1}$ at each temperature. At lower algal concentrations, however, there was a general trend of increased mortality with a reduction in food supply and this was enhanced with a rise in temperature from 15°C to 25°C. Significant effects (Fisher's Exact Test, $P < 0.05$) of food concentration on copepod survival were observed at algal concentrations of 83 $\mu gC\ l^{-1}$ at 15 and 20°C, and at algal concentrations up to and including 520 $\mu gC\ l^{-1}$ at 25°C (Table 2.2).

2.3.2. Development

The stage durations for copepods reared at the different temperature and food concentrations are shown in Table 2.3 and Figure 2.1. The effect of these variables on postembryonic development was compared using the stage duration times for the combined naupliar and copepodid stages (N1-N6 and C1-C5), and the total development time from hatching to adult (N1-Adult). Food concentration and temperature had a significant effect on development times (Two-way ANOVA, $P < 0.01$), and the lack of interaction between food concentration and temperature indicated that these variables were acting independently (Table 2.4).

2.3.3. Effect of food concentration on development

At each temperature (15, 20 and 25°C), the development times for copepods exposed to algal concentrations of 1300 and 3250 $\mu gC\ l^{-1}$ were similar, however, at lower algal concentrations

(83, 208 and 520 μgC l^{-1}) it took significantly longer for copepods to reach adulthood (Table 2.5). For example, copepods took between 4.8 and 6.2 days longer to reach adulthood following a reduction in algal concentration from 3250 μgC l^{-1} to 520 μgC l^{-1} (6.25 times less algae). Copepod mortality in algal concentrations lower than 520 μgC l^{-1} was high, and increases in development time for surviving copepods were judged to be significant without the need for statistical analysis. Out of the total number of copepods (n = 60) exposed to algal concentrations of 83 and 208 μgC l^{-1}, 58.3% (n = 35) died during development, 23.3% (n = 14) failed to develop beyond the C3-C5 copepodid stages after 28 days (end of experiment), and only 18.3% (n = 11) successfully developed to adulthood. The small number (n = 11) of surviving copepods at 83 and 208 μgC l^{-1} took approximately twice as long to reach adulthood compared with copepods exposed to 3250 μgC l^{-1} (Table 2.3)

2.3.4. Effect of temperature on development

At each algal concentration, there was a general trend for a reduction in copepod development times in response to increasing temperature over the range 15 - 25°C (Table 2.3). At algal concentrations of 1300 and 3250 μgC l^{-1}, development times became significantly shorter as the temperature increased from 15 to 25°C (Table 2.6). For example, adult development time at a concentration of 3250 μgC l^{-1} declined from 12.4 days at 15°C to 8.8 days at 20°C and 7.3 days at 25°C. At 520 μgC l^{-1}, it took significantly longer for copepods to reach the adult stage at 15°C (17.6 days) compared with 20 and 25°C (13.6 and 13.5 days respectively); differences in development time between 20 and 25°C were not significant (Table 2.6). Comparison of development times at algal concentrations lower than 520 μgC l^{-1} (83 and 208 μgC l^{-1}) was difficult as mortality in these treatments was significant (40% in 208 μgC l^{-1} and 77% in 83 μgC l^{-1}) and few copepods successfully reached the adult stage. Between temperature comparison of adult development times at a concentration of 208 μgC l^{-1}, for which only 30% (n = 9) of the copepods

successfully reached adulthood, revealed a significant difference in adult development time at the extremes (15 and 25°C) of the temperature range (Table 2.6).

2.4. DISCUSSION

Data on the duration of the larval stages of marine harpacticoids and the influence of environmental factors (and their potential interaction) on postembryonic development are relatively limited. A compilation of temperature-related development times for marine harpacticoids in laboratory culture (28 species, including 6 *Tisbe* spp) was provided by Hicks & Coull (1983), who described development times for the combined naupliar stages and time from hatching to adult. Individual stage durations for a relatively small number of marine harpacticoids (6 species, including 1 *Tisbe* sp.) were described by Bergmans (1981a). Comparison of the development times for *Tisbe battagliai* with these literature values must be made with caution, however, as differences may be explained by a number of factors including the feeding regimes used, previous culture history and genetic differences between populations. Even so, it is clear that postembryonic development rates of harpacticoids are strongly temperature dependant, and development times decrease with increasing temperature (Hicks & Coull, 1983). This temperature trend is shown in the present study and suggests that seasonal temperature changes play an important role in the population dynamics of *T. battagliai* and other harpacticoid species; fastest development is likely to occur in the warmer months of the year when the availability of preferred, or nutritionally superior, food may also be high. Development times at temperatures lower than 15°C (not investigated in this study) would be expected to increase, particularly at low food concentrations.

An extensive review of postembryonic durations of both marine and freshwater copepods was provided by Hart (1990) who suggested the relationship between total copepodid and naupliar

duration (Dc/Dn ratio) might provide a useful and simple index of food availability during development. While both naupliar and copepodid stages require food for maintenance and growth, the data examined by Hart (1990) showed, with few exceptions, the enhancement of naupliar development with rising food supply was far less pronounced than the corresponding acceleration of copepodid development with equivalent food increases. The results for *Tisbe battagliai* revealed a similar relationship to that described by Hart (1990) for the influence of food concentration on naupliar and copepodid development. The Dc/Dn ratio for *T. battagliai* was inversely related to food concentration, however, a relationship between Dc/Dn ratio and temperature was less clear (Fig. 2.2). Over the temperature range 15 - 25°C, declining food concentration had a more pronounced effect on copepodid, compared with naupliar, development and changes in the Dc/Dn ratio were attributed to prolonged development of the copepodid stages at low food concentrations. For example, a reduction in algal concentration from 3250 to 520 $\mu gC\ l^{-1}$ significantly increased the combined development times by between 0.7 and 1.5 days for the naupliar, and between 4.1 and 4.7 days for the copepodid stages. Development times were calculated only for those copepods that reached the adult stage. However, at 15 and 20°C, a large proportion (59%) of the surviving copepods at 83 and 208 $\mu gC\ l^{-1}$ remained in the copepodid stages after 28 days (end of experiment) and were, therefore, not included in the calculation of mean development times. Consequently, the values of development times at these combinations of temperature (15 and 20°C) and low algal concentration (83 and 208 $\mu gC\ l^{-1}$) underestimate the effect of these variables on development. Data for all surviving copepods (including values for those copepods that did not reach the adult stage) indicate that the combined copepodid durations were between 7.2 and > 21 days longer (compared with 7.2 - 10.8 d for only those that reached the adult stage) than the equivalent development times for copepods reared at an algal concentration of 3250 $\mu gC\ l^{-1}$.

The relative independence of the nauplii to low food concentrations may be attributed partly to the relatively low metabolic rate and energetic efficiency of the naupliar form at small body sizes

(Epp & Lewis, 1980). Even in treatments containing no algal food (0 $\mu gC\ l^{-1}$), nauplii were able to survive for several days before the onset of mortality which occurred after 3 - 7 days at 20 and 25°C and after 9 - 12 days at 15°C. Literature data to support the concept of non-feeding naupliar stages could not be found. It appears that most harpacticoid nauplii have well developed mandibles and the naupliar antenna (2nd antenna) differs from the same structure in copepodid stages in that it has special adaptations for mastication. Although they do not have a fully developed gut, such structural adaptations suggest that harpacticoid naupliar stages are capable of taking in food (J.M. Gee, *pers. comm.*). It is worth noting that nauplii of the cyclopoid copepod *Oithona ovalis* have a functioning gut (Fanta, 1976). Experimental conditions did not preclude the growth of bacteria or presence of dissolved organic matter which, in addition to the algae added as food, may have provided additional sources of nutrition for the early life stages. *Tisbe* spp are capable of utilising a wide range of different food sources (Hicks & Coull, 1983), of which bacteria have been shown to be important dietary components for some species (Brown & Sibert, 1977; Rieper, 1978; Vanden Berghe & Bergmans, 1981). Since harpacticoids are not generally known to store lipids as an energy source during or prior to reproduction (as in many calanoids), use of recently ingested food is assumed to be the basis for energetic contribution to eggs (Hicks & Coull, 1983). In this present study, experiments were started with nauplii derived from well fed culture females, therefore, it is possible that maternal investment in energy resources of the egg may have contributed to naupliar survival in the food-limited treatments.

Coull & Dudley (1976) observed delayed naupliar development in four species of sediment-dwelling harpacticoids, possibly in response to resource limitation, however, there is little evidence of this response for phytal harpacticoids. In the present study, low food concentrations appeared to increase mortality of the naupliar stages and prolong development of the surviving copepodid stages. Hicks (1979) suggested that prolonged juvenile harpacticoid development may serve to limit potential over exploitation of food resources and thereby prolong survival in temporarily food-

limited environments. The phytal biotype, occupied by some species of *Tisbe*, however, offers a potentially continuous and abundant supply of food (Hicks, 1977, 1979), although there is no information on the seasonal variability of available food resources or of its nutritional value to the different copepod life stages. Consequently, seasonal availability of food may be an important factor controlling the development of phytal-dwelling harpacticoids and changes in juvenile development may reflect a response to environmental cues (e.g. food limitation).

Predation pressure may also influence life-history traits and, in habitats where predation does occur, acceleration of naupliar development may increase the chances of survival when risks to the naupliar stages are high. Supporting evidence for this hypothesis is, however, limited and the predators of phytal harpacticoids have not been defined adequately (Hicks, 1980). Selective predation by juvenile fish on the naupliar stages of the harpacticoid *Parastenhelia megarostrum* was reported by Hicks (1984) and naupliar mortality in field populations of the meiobenthic species *Microarthridion littorale* was related to juvenile fish predation (Morris & Coull, 1992).

It is extremely difficult to extrapolate from the laboratory to the field situation where food resources, and their utilisation by different species of harpacticoid, are characterised by complex trophic relationships (Lee *et al.*, 1976; Hicks & Coull, 1983). It is not known whether the stage durations observed in this study are optimal, as potential nutritional deficiencies in the algal diet, and genetic changes due to inbreeding depression in the cultures, may have caused changes in copepod fitness traits (e.g. development rate) with time. Although many species of copepods can be reared successfully on single species algal diets (Paffenhofer & Harris, 1979; Arnott *et al.*, 1986), it is now well established that the reproductive performance and health of copepod populations in the laboratory are favoured by mixed foods, reflecting the need for a balanced diet (Hicks & Coull, 1983). At the Brixham Environmental Laboratory, cultures of *Tisbe battagliai* have been maintained successfully on a unialgal diet of *Isochrysis galbana* for several years, however, the culture conditions do not exclude bacteria or dissolved organics which may provide additional

sources of food. For example, bacteria have been shown as a potential food source for naupliar and adult stages of the harpacticoid *Nitocra lacustris* (Decho & Fleeger, 1988) and *N. spinipes* (Weiss *et al.*, 1996). In the later study, 24% of the original nauplii attained the copepodid stage without any form of particulate food and the authors suggested that the nauplii may have assimilated dissolved organic carbon. In the present study, *I. galbana* may not have provided optimal nutrition, therefore, the quantity of algae added as food may not have been sufficient for the different copepod life stages.

While temperature is certainly a major factor controlling the development of many harpacticoids, food availability may be a significant factor superimposed on any temperature influence (Hicks, 1979). Food limitation had a pronounced effect on postembryonic development of *Tisbe battagliai* and prolonged stage durations were observed at relatively high algal concentrations (520 $\mu gC\ l^{-1}$). This may be due partly to potential nutritional deficiencies in the unialgal diet, however, it remains to be seen whether similar delays in development occur for copepods fed the same algal concentrations, but of a mixed species diet. The review of copepod postembryonic durations by Hart (1990) focused mainly on marine and freshwater calanoids, and these data implied that growth-saturating food concentrations were perhaps lower in marine than freshwater calanoids (circa 100 compared with circa 650 $\mu gC\ l^{-1}$). Data describing the effect of food concentration on postembryonic durations in marine harpacticoids are limited, and further investigations are required to assess whether threshold food concentrations for development occur at similar concentrations to those described above for marine and freshwater calanoids.

Table 2.1. Preparation of marine algal culture medium (with EDTA).

SOLUTION A	
$NaNO_3$	100.00g
$NaH_2PO_4.2H_2O$	20.00 g
$FeCl_3.6H_2O$	1.30 g
$MnCl_2.4H_2O$	0.36 g
H_3BO_3	33.60 g
$Na_2EDTA.2H_2O$	45.00 g
Trace metal solution	1.0 ml
Distilled water to	1 litre
SOLUTION B	
Vitamin B_{12}	0.02 g
Vitamin B_1 (Thiamine)	0.40 g
Distilled water to	100 ml
TRACE METAL SOLUTION (for preparation of Solution A)	
$ZnCl_2$	2.10 g
$CoCl_2.6H_2O$	2.00 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	0.90 g
$CuSO_4.5H_2O$	2.00 g
Distilled water to	100 ml
Acidify drop-wise with N/10 HCl until clear)	

Table 2.2. Mortality (%) of *Tisbe battagliai* recorded during naupliar and copepodid development. Values in parentheses denote days on which mortalities occurred. 10 copepods were tested per concentration. † indicates a significant effect ($P < 0.05$) on copepod survival compared with those treatments exposed at the same temperature showing no mortality.

Temp. (°C)	Algal conc. (µgC l^{-1})	% Mortality		Total mortality (%)
		Nauplius	Copepodid	
15	3250	0	0	0
	1300	0	0	0
	520	0	0	0
	280	10 (9)	0	10
	83	20 (12)	20 (11+16)	40 †
	0	80 (9-12)	0	80 †
20	3250	0	0	0
	1300	0	0	0
	520	0	0	0
	280	20 (9+10)	10 (27)	30
	83	90 (4-9)	10 (11)	100 †
	0	100 (4+5)	0	100 †
25	3250	0	0	0
	1300	0	0	0
	520	0	40 (7-19)	40 †
	280	70 (3-6)	10 (6)	80 †
	83	80 (4-6)	10 (7)	90 †
	0	90 (3-7)	10 (10)	100 †

Table 2.3. Development times (days) of combined naupliar stages (N1-N6), individual and combined copepodid stages (C1-C5), and hatching to adult (N1-A) for *Tisbe battagliai* reared at different algal concentrations at three temperatures (values represent mean ±1SD). n = number of survivors (out of 10) at the end of the experiment (see Table 2.2), n^a = number of survivors that developed to the adult stage within 28 days (end of experiment).

Algal conc. ($\mu gC\ l^{-1}$)	Temp. (°C)	n	n^a	Naupliar stages (N1-N6)	Individual copepodid stages					Copepodid stages (C1-C5)	Hatching to adult (N1-A)
					C1	C2	C3	C4	C5		
3250	15	10	10	5.2 ±0.42	1.80 ±0.63	1.00 ±0	1.30 ±0.48	1.20 ±0.42	1.90 ±0.74	7.20 ±0.92	12.4 ±0.70
	20	10	10	4.0 ±0	0.60 ±0.21	0.60 ±0.21	1.00 ±0.41	0.90 ±0.21	1.70 ±0.48	4.80 ±0.42	8.80 ±0.42
	25	10	10	3.0 ±0	0.85 ±0.24	0.60 ±0.21	0.85 ±0.47	0.95 ±0.16	1.05 ±0.37	4.30 ±0.48	7.30 ±0.48
1300	15	10	10	5.4 ±0.52	1.80 ±0.63	1.00 ±0	1.20 ±0.42	1.60 ±0.52	2.60 ±0.70	8.20 ±1.23	13.6 ±1.26
	20	10	10	4.0 ±0	1.00 ±0	1.00 ±0	0.95 ±0.16	1.05 ±0.37	1.80 ±0.42	5.80 ±0.42	9.80 ±0.42
	25	10	10	3.0 ±0	1.05 ±0.37	0.85 ±0.24	0.90 ±0.21	1.10 ±0.32	1.10 ±0.32	5.00 ±0.47	8.00 ±0.47
520	15	10	10	6.3 ±0.67	1.90 ±0.57	1.50 ±0.53	2.00 ±0.67	2.10 ±1.20	3.80 ±1.14	11.3 ±2.11	17.6 ±2.01
	20	10	10	4.7 ±0.48	1.30 ±0.67	1.40 ±0.52	1.10 ±0.32	1.60 ±0.52	3.50 ±2.01	8.90 ±2.77	13.6 ±2.84
	25	6	6	4.5 ±1.08	1.25 ±0.46	1.13 ±0.35	1.38 ±0.74	2.75 ±1.91	2.67 ±0.82	9.17 ±4.02	13.5 ±4.37
208	15	9	2	7.5 ±0.71	2.00 ±0	2.50 ±0.71	3.00 ±0	5.00 ±0	5.50 ±0.71	18.0 ±1.41	25.5 ±2.12
	20	7	5	6.2 ±1.10	2.20 ±0.45	1.80 ±0.84	2.60 ±1.14	3.40 ±1.52	5.00 ±1.41	15.0 ±3.54	21.2 ±3.19
	25	2	2	4.5 ±0.71	1.00 ±0	2.00 ±0	1.50 ±0.71	3.50 ±0.71	3.50 ±0.71	11.5 ±2.12	16.0 ±2.83
83	15	6	2	8.5 ±0.71	3.00 ±0	2.50 ±0	3.00 ±0	3.50 ±0.71	5.50 ±0.71	17.5 ±2.12	26.0 ±1.41
	20	0	0	-	-	-	-	-	-	-	-
	25	1	0	-	-	-	-	-	-	-	-

Table 2.4. Two-way analysis of variance (ANOVA) of the effect of temperature (15, 20 and 25°C) and food (algal concentrations of 208, 520, 1300 and 3250 µgC l^{-1}) on the development time (days) of *Tisbe battagliai* (n.s., not significant; ** significant at the 1% level).

Source of variation	d.f.	Sum of squares	Mean square	F-ratio	Significance level
NAUPLIAR STAGES (N1-N6)					
Temperature	2	55.8	27.9	119.8	**
Algae	3	37.2	12.4	53.2	**
T x A	6	2.3	0.4	1.6	n.s.
Error	83	19.3	0.2		
Total	94	146.8			
COPEPODID STAGES (C1-C5)					
Temperature	2	140.9	70.5	21.3	**
Algae	3	698.7	232.9	70.3	**
T x A	6	18.9	3.1	0.9	n.s.
Error	83	274.8	3.3		
Total	94	1257.3			
HATCHING TO ADULT (N1-A)					
Temperature	2	372.1	186.0	54.4	**
Algae	3	1055.4	357.8	102.9	**
T x A	6	32.1	5.3	1.6	n.s.
Error	83	283.7	3.4		
Total	94	1997.3			

Table 2.5. Results of one-way analysis of variance (ANOVA) followed by *t*-tests (two-tailed) of the effect of algal concentration on mean development times of *Tisbe battagliai* at different temperatures (n.s., not significant; ** significant at the 1% level). Combined stage durations at algal concentrations lower than 520 μgC l^{-1} were judged to be significant without the need for statistical analysis (see text).

Combined stage durations	Temp. (°C)	Algal concentration (μgC l^{-1})	Significance level
Naupliar (N1-N6), copepodid (C1-C5) and hatching to adult (N1-A)	15	1300 vs 3250	n.s.
		520 vs 1300	**
		520 vs 3250	**
Naupliar (N1-N6), copepodid (C1-C5) and hatching to adult (N1-A)	20	1300 vs 3250	n.s.
		520 vs 1300	**
		520 vs 3250	**
Naupliar (N1-N6), copepodid (C1-C5) and hatching to adult (N1-A)	25	1300 vs 3250	n.s.
		520 vs 1300	**
		520 vs 3250	**

Table 2.6. Results of one-way analysis of variance (ANOVA) followed by *t*-tests (two-tailed) of the effect of temperature on mean development times of *Tisbe battagliai* at different food concentrations (n.s., not significant; * significant at the 5% level; ** significant at the 1% level).

Combined stage durations	Algal conc. ($\mu gC\ l^{-1}$)	Temperature (°C)	Significance level
Naupliar stages (N1-N6)	1300 & 3250	15 vs 20 vs 25	**
	520	15 vs 20	**
	520	15 vs 25	**
	520	20 vs 25	n.s.
	208	15 vs 20	n.s.
	208	20 vs 25	n.s.
	208	15 vs 25	**
Copepodid stages (C1-C5)	3250	15 vs 20	**
	3250	15 vs 25	**
	3250	20 vs 25	n.s.
	1300	15 vs 20	**
	1300	15 vs 25	**
	1300	20 vs 25	n.s.
	520	15 vs 20 vs 25	n.s.
	208	15 vs 20 vs 25	n.s.
Hatching to adult (N1-A)	1300 & 3250	15 vs 20 vs 25	**
	520	15 vs 20	*
	520	15 vs 25	*
	520	20 vs 25	n.s.
	208	15 vs 20	n.s.
	208	20 vs 25	n.s.
	208	15 vs 25	*

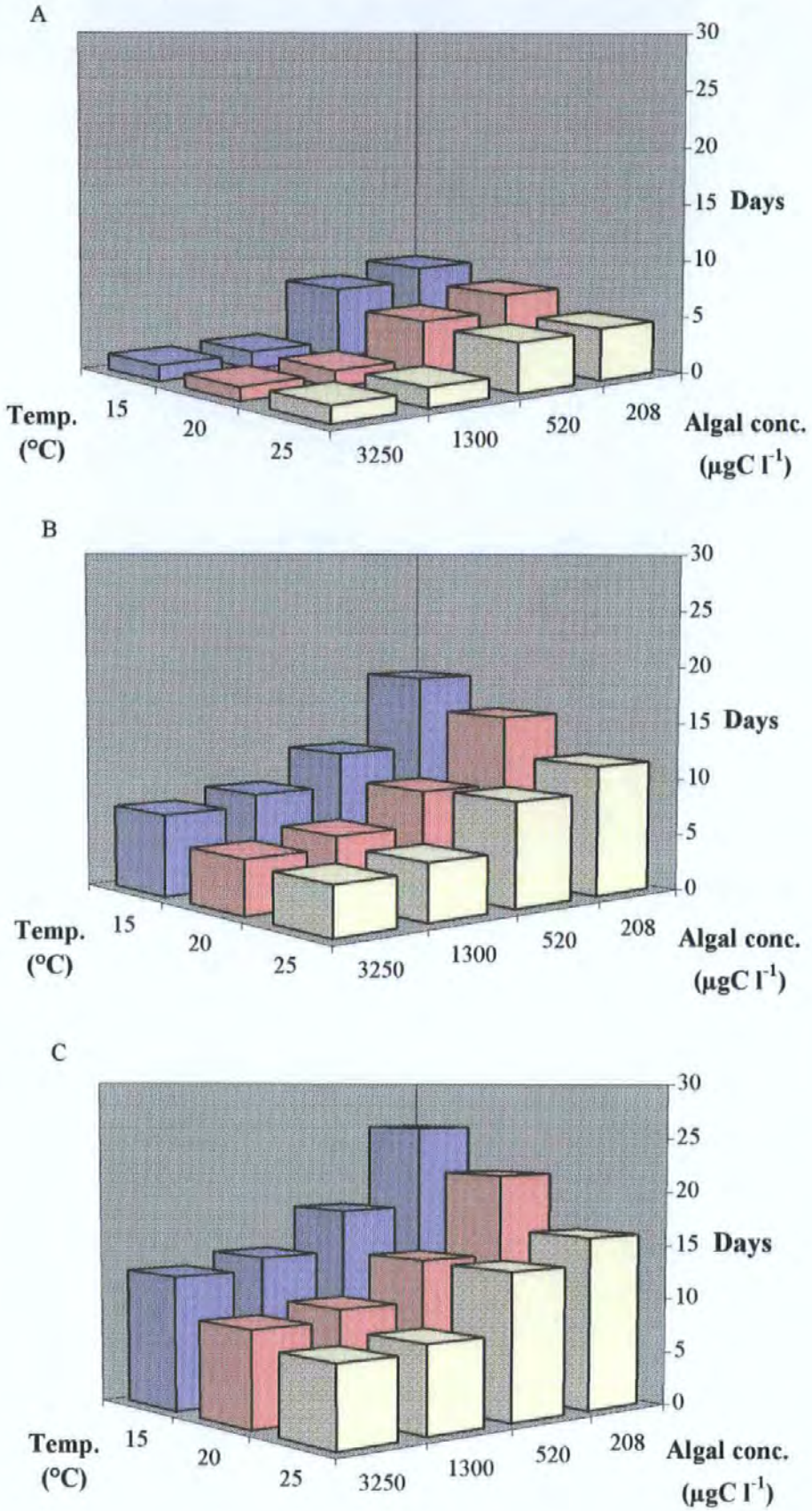


Figure 2.1. The effect of temperature and food concentration on mean development times of (A) combined naupliar stages, (B) combined copepodid stages, and (C) time from hatching to adult for *Tisbe battagliai*. Variation around the mean values is shown in Table 2.3.

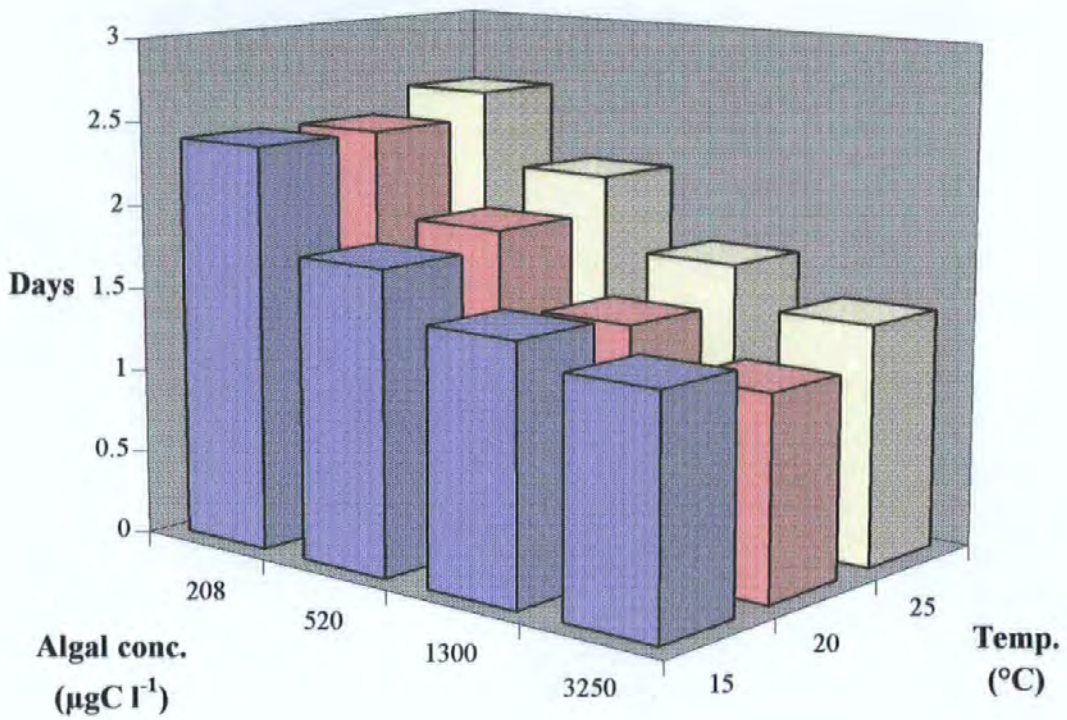


Figure 2.2. The influence of temperature and food concentration on the ratio of combined copepodid and naupliar development times (Dc/Dn ratio) for *Tisbe battagliai* (mean values). Data are taken from the mean development times shown in Table 2.3.

CHAPTER 3

EFFECTS OF TEMPERATURE AND FOOD QUANTITY ON THE REPRODUCTION OF *TISBE BATTAGLIAI* (COPEPODA: HARPACTICOIDA)

This Chapter was presented as a paper at the European Marine Biology Symposium, held at the University of Southampton, September 18-22, 1995.

3.1. INTRODUCTION

Harpacticoid copepods can dominate the sediment and phytal meiobenthos of marine ecosystems (Hicks & Coull, 1983). Some species form an important food source for the small (30 - 60 mm) juveniles of some commercially important fish species (Gee, 1989), making harpacticoids potentially valuable live food organisms for feeding marine fish larvae in culture (Van der Meeren, 1991; Norsker & Stottrup, 1994). Harpacticoids are sensitive to contaminants (Lassus *et al.*, 1984; Bengtsson & Bergstrom, 1987; Chandler & Scott, 1991; Strawbridge *et al.*, 1992; Hutchinson *et al.*, 1994) and have been used to assess the effects of anthropogenic disturbance on aquatic ecosystems (Coull & Chandler, 1992). The growing interest in the use of harpacticoids as marine bioassay organisms is frequently limited, however, by inadequate knowledge of the influence of environmental factors on their development and reproduction. Whilst temperature is a major factor controlling reproductive activity in benthic harpacticoids, food availability may also have a significant effect on their reproduction (Hicks, 1979), yet details are lacking for many species. The suitability of members of the *Tisbe* genus as laboratory test organisms has been reported previously (Battaglia, 1970; Williams, 1992; Williams *et al.*, 1993). Members of the genus *Tisbe* occur normally as multispecies guilds, often including several members of a single group of sibling species (Bergmans & Janssens, 1988); *Tisbe battagliai* is a sibling species of the *"holothuriae"* group. Knowledge of the biology of *T. battagliai* is limited to laboratory studies of food preference (Vanden Berghe & Bergmans, 1981), the influence of salinity on the life history of *T. battagliai* and *T. holothuriae* in pure and mixed cultures (Bergmans & Janssens, 1988), and the influence of temperature and food concentration on postembryonic development (Williams & Jones, 1994).

The aim of this chapter was to establish the combined effect of temperature and food concentration on aspects of the reproduction of *Tisbe battagliai*. Such information is not only valuable in defining the conditions most favourable for the mass culture of this species (for example,

see papers by Miliou & Moraitou-Apostolopoulou, 1991abc, and Miliou, 1992 for *T. holothuriae*) but adds also to an understanding of the influence of environmental factors on harpacticoid reproduction.

3.2. MATERIALS AND METHODS

3.2.1. Copepod maintenance

Experimental cultures of *Tisbe battagliai* were maintained in filtered (0.2 µm), natural seawater (approx. salinity of 35‰) obtained from Tor Bay, Devon, and held in constant temperature rooms at 15, 20 and 25°C under a photoperiod of 16 h light: 8 h dark. Culture vessels (rectangular polystyrene tanks: 25 cm long, 15.5 cm wide and 15 cm tall) contained 3 l of seawater which was replaced once per week with freshly filtered seawater which had been aged for 7 days prior to filtration. Copepods were fed the prymnesiophyte *Isochrysis galbana* Parke (Strain CCAP 927/1, Culture Centre of Algae and Protozoa) grown in a chemostat using the culture methods described in the previous chapter (Williams & Jones, 1994). The harvested algae were centrifuged (5 K for 15 mins) and, after discarding the supernatant, the algal cells were resuspended in filtered (0.2 µm) seawater to provide a stock suspension containing 5 x 10^7 cells ml^{-1}. Initially, cultures were fed 5 ml l^{-1} (equivalent to 2.5 x 10^5 cells ml^{-1} in 3 l) of the algal stock suspension once per week (when the seawater was renewed); a similar quantity of algae was added daily, provided the animals had cleared the previous days food suspension from the water.

3.2.2. Test procedure

Harpacticoid copepods reproduce sexually and males transfer sperm to the female in spermatophores (Hicks & Coull, 1983). Spermatophore-mediated sperm transmission is very efficient and, from a single copulation, female harpacticoids are capable of using stored sperm to

fertilise successive broods of eggs throughout their lifespan. Eggs are usually carried in a single or paired external egg sacs and, after a period of embryonic development, hatch into the naupliar stage. Copepod reproduction provides a potentially sensitive indicator of response to environmental variables. In this study, the progress of reproduction in individual females was followed from the time of production of the first egg sac through to day at death.

Experiments were carried out in temperature-controlled rooms (± 1°C) with a photoperiod of 16 h light: 8 h dark. From the time of first egg sac production, adult females were reared at three temperatures (15, 20 and 25°C) and six concentrations of *Isochrysis galbana* (0 - 3250 µgC l^{-1}). The temperatures investigated in this study covered the upper seasonal range experienced by *Tisbe battagliai* in British and Mediterranean coastal waters (Williams & Jones, 1994). The algal concentrations were selected to cover the range of 0 (no algal food) to 3250 µgC l^{-1}, the highest algal concentration has previously been observed to support high offspring production in *T. battagliai* (*pers. obs.*). Adult females were from a cohort of nauplii (< 24 h old) reared in cultures maintained at the appropriate test temperature until the first egg sacs were visible (the time periods were 16, 14 and 12 days for females reared at temperatures of 15, 20 and 25°C respectively). To limit the transfer of algal cells, ovigerous females were sieved carefully onto a 180 µm nylon mesh and resuspended in freshly filtered seawater before being placed in multi-well, disposable, polystyrene tissue culture plates with close fitting lids. Stock suspensions of *I. galbana* were diluted with filtered (0.2 µm) seawater to provide concentrations of 0, 83, 208, 520, 1300 and 3250 µgC l^{-1}; each algal concentration increasing stepwise by a factor of 2.5. Corresponding values (mean measured), based on algal cell numbers, ranged from approximately 0.1 - 4.5 x 10^5 cells ml^{-1} (83 - 3250 µgC l^{-1}). Carbon values and cell concentrations for the freshly prepared algal stock suspensions were determined using a Dohrman DC-190 high temperature carbon analyser and a Model ZB Coulter Counter respectively. Algal stock suspensions were stored in a refrigerator in the dark and used within 7 days. Ten females were maintained at each combination of temperature

and food concentration until death. Individual females were added randomly, using a pasteur pipette, to 10 wells of tissue culture plates, each containing 5 ml of test solution. Transfers and observations were carried out under a Wild M8 binocular stereomicroscope, using darkfield illumination. Observations of mortality (defined as absence of any movements by the organism when examined by microscope for a period of 15 secs), the total number of broods and the total number of offspring produced by each female were made at 24-h intervals. From these measurements, female lifespan [defined as the number of days between production of the first egg sac (start of experiment) and day at death], the number of female reproductive days (defined as the number of days between hatching of the first and last broods of offspring), the number of offspring per brood and the interbrood duration (defined as the number of days between hatching of successive broods) were calculated. Surviving copepods were transferred daily to duplicate test chambers containing freshly prepared test solutions at the appropriate algal concentration. In each treatment, temperature was measured daily, pH and dissolved oxygen concentration at least weekly. Experiments were started with ovigerous females derived from laboratory cultures, therefore, production of the first brood of offspring was judged to have been influenced by the previous culture regime. Consequently, analysis of the total number of broods and total number of offspring produced excluded first brood values.

Copepods that failed to produce offspring beyond the first brood (mainly confined to algal concentrations lower than 1300 $\mu gC\ l^{-1}$) were classed as non reproductive and excluded from any analysis. Copepods did not remain reproductive throughout their lifespan and, after release of their final brood of offspring, survived for several days without further reproduction. To provide a comparison between total offspring production and the rate of reproduction at different temperature regimes, reproduction was also expressed as the daily offspring production during the period of time each female was reproductive (the number of offspring per female per reproductive day). These results should be treated with caution, however, since an animal that produced a small number of

offspring over a short time period can provide a similar value for reproduction as an animal that produced a relatively large number of offspring over a longer period of time.

3.2.3. Statistical analysis

Female lifespan and reproductive parameters were tested for normality and equality of variances prior to analysis of variance techniques (Sokal & Rohlf, 1995). Data that failed to meet the assumptions for analysis of variance (ANOVA) were analysed with non parametric (distribution-free) techniques (Steel, 1959; Hollander & Wolfe, 1973). Food concentrations lower than 520 µgC l^{-1} were excluded from the analyses of reproductive parameters due to limited reproduction in these treatments.

3.3. RESULTS

3.3.1. Lifespan

Temperature and food concentration had significant independent and interactive effects on female lifespan (Table 3.1). Female lifespan was inversely related to temperature (Table 3.2) and significant reductions in lifespan were observed with increases in test temperature over the range 15 - 25°C (Table 3.3). For example, mean lifespan for females reared at an algal concentration of 3250 µgC l^{-1}, ranged from 53 days at 15°C to 31 days at 20°C and 23 days at 25°C. Effects were most pronounced over a 10°C range in temperature and, at the range of algal concentrations tested (83 - 3250 µgC l^{-1}), females reared at 15°C lived approximately twice as long as those reared at 25°C. Lifespan was more sensitive to reductions in food concentration for females reared at 15 and 25°C than those reared at 20°C (Table 3.2). Compared with the highest algal concentration tested (3250 µgC l^{-1}), there was a significant shorter lifespan at lower algal concentrations (1300 & 520 µgC l^{-1})

at 15 and 25°C. There was no significant effect (reduction) on lifespan over a 16 fold range in algal concentrations (208 - 3250 μgC l^{-1}) at 20°C (Table 3.2).

3.3.2. Reproduction

Temperature and food concentration had significant independent and interactive effects on the measured reproductive parameters (Table 3.1). As noted above, these variables had a significant effect on female lifespan, and length of life was expected to be an important determinant of reproductive output. Females did not remain reproductive throughout their lifespan and the reproductive period of individual females, expressed as the total number of female reproductive days, was used to calculate the rate of reproduction (number of offspring per female per reproductive day) at the different temperature and food concentrations (Table 3.4).

Temperature had a significant effect on the number of broods and number of offspring produced by *Tisbe battagliai* (Table 3.5). Provided that sufficient food was available (e.g. 3250 μgC l^{-1}), the optimal temperature for offspring production in this study was 20°C (Fig. 3.1). At this temperature, individual females fed algal concentrations of 3250 μgC l^{-1} produced an average of 6.7 broods (range of 5 - 9) and 217 offspring (range of 170 - 281). The latter were produced at a rate of 10 offspring per female per reproductive day (Table 3.4). By comparison, temperature had no effect on the total numbers of offspring produced by females reared at 15°C but there was a significant reduction in the numbers of offspring per female per reproductive day from 10 to 5.7 (Table 3.5). These differences in the rate of reproduction at 15 and 20°C may be explained by the longer time interval between the release of successive broods at the lower temperature (Table 3.7). At 25°C, the rate of offspring production by females fed the high ration (3250 μgC l^{-1}) was very similar to that at 20°C (9.6 and 10.0 respectively). There was, however, a significant reduction in the total numbers of offspring produced at 25°C compared with the total numbers of offspring produced at 15 and 20°C. The reduction in total offspring production at 25°C was attributed to the

relatively large reduction in lifespan and reproductive period at this temperature (Tables 3.2 and 3.4 respectively). There was no clear effect of temperature on brood size, however, results did suggest a general reduction in brood size at 25°C compared with those at 15 and 20°C (Table 3.6). The time interval between hatching of successive broods increased with each reduction in temperature from 25 - 15°C (Table 3.7). A decrease in temperature from 25 to 15°C (a range of 10°C) caused an approximate doubling of the interbrood duration (hatching to hatching).

The reproductive parameters measured were very sensitive to changes in food concentration, and a relatively small decrease in algal concentration resulted in significant reductions in the number of broods and the numbers of offspring produced (Table 3.8; Fig. 3.2). For example at 15 and 20°C, a reduction in algal concentration from 3250 to 1300 $\mu gC\ l^{-1}$ (2.5 times less algae) resulted in a significant decrease in all the measured reproductive parameters (total number of broods, total number of offspring and the number of offspring per female per reproductive day). Effects of food concentration were less pronounced at 25°C and a reduction in algal concentration from 3250 to 520 $\mu gC\ l^{-1}$ (6.25 times less algae) was required for a significant reduction in all the measured reproductive parameters (Table 3.8). At each temperature, increases in algal concentration over the range 520 - 3250 $\mu gC\ l^{-1}$ were associated with an increase in brood size (Table 3.6) and a reduction in the interval of time between production of successive broods (Table 3.7). For example at 20°C, a reduction in algal concentration from 3250 to 1300 $\mu gC\ l^{-1}$ resulted in a 1.6 to 4.0 times reduction in the numbers of offspring per brood and an increase in the time interval between the release of successive broods of between 1.0 to 3.7 d. Few copepods at the lower ration (1300 $\mu gC\ l^{-1}$) produced more than five broods hence the comparisons above were made only for broods 2 - 5.

3.4. DISCUSSION

Present results show that temperature and food concentration (acting independently and in combination) have significant effects on brood size and the frequency of brood production of *Tisbe battagliai*. The general trends reported are in accord with other studies of marine harpacticoids (Hicks & Coull, 1983) and for marine (Klein Breteler & Schogt, 1994) and freshwater calanoids (Jamieson & Burns, 1988).

Temperature exerted a significant influence on reproduction through effects on the duration of female lifespan and reproductive period, and through changes in the frequency of brood production. Temperature has a major effect on metabolic rate and, at suboptimal temperatures, higher maintenance costs may reduce the level of investment in reproduction. In this study, the optimum temperature for offspring production was 20°C and potential increases in the metabolic costs required for maintenance at 15 and 25°C may explain partly why fewer offspring were produced at these temperatures. A temperature of 25°C may also be approaching an upper threshold for *Tisbe battagliai* and temperature stress may have negative effects on survival and fecundity. The effects of temperature on reproductive output of *T. battagliai* support the trend described by Miliou & Moraitou-Apostolopoulou (1991a) for the Greek strain of *T. holothuriae*, where the most favourable conditions for development and reproduction were 19°C and 38 ‰; at lower or higher temperatures (14 and 24°C), the number of egg sacs and total number of offspring decreased. More recently, Heath (1994) reported 20°C and 30‰ as the optimum environmental conditions for rearing a strain of *T. holothuriae* from the West Coast of Scotland. These same temperature and salinity optima were reported for rearing a strain of *T. holothuriae* from Helgoland (Hoppenheit, 1975).

The influence of diet on the reproductive parameters of *Tisbe* spp cultured in the laboratory has been investigated by several authors (Heath, 1994 and references cited therein). Different food

types appear to elicit different reproductive responses but the specific components of the diet responsible for these differences have not been identified or are poorly understood. Under laboratory conditions, harpacticoids ingest a wide variety of foods, however, this may reflect laboratory adaptability rather than selection for an optimal food resource (Hicks & Coull, 1983). In laboratory studies, offspring production generally declines with reduction in food supply below optimal levels, however, high food concentration does not necessarily ensure adequate food quality. Miliou & Moraitou-Apostolopoulou (1991b) measured the effect of seven diets on the population dynamics of *Tisbe holothuriae* reared under constant conditions (19°C, 38‰). All life-cycle parameters were affected by the type of food offered, and reproductive performance was most efficient for copepods fed a mixed diet of seaweed (*Ulva* sp.) and fryfood (a commercial liquid product containing animal and vegetable components and enriched with vitamins). The reproductive output of copepods fed on this diet was generally low (maximum of 3 egg sacs and 90 offspring per female) compared with other published values for *T. holothuriae*, and larval mortality during development was very high (41 - 80% after 10 days) in all dietary treatments. These results suggest that the diets used by Miliou & Moraitou-Apostolopoulou (1991b) may not have been optimal for development or reproduction. Gaudy & Guerin (1977) reared *T. holothuriae* on 3 artificial foods (Tetramin, Bioter and Renutryl) under constant conditions (19°C, 38‰) in the laboratory. These authors reported that lifespan (including larval stages) ranged from 24 - 33 days, reproductive period from 7 - 11 days, and copepods produced an average of 4 - 5 egg sacs (51 - 69 eggs per sac) and 258 - 416 nauplii per female. The latter results were compared with reproductive parameters for 6 other *Tisbe* spp reared in the laboratory on microalgal diets at temperatures ranging from 17 - 19°C; measured values ranged from 24 - 81 days for longevity, and females produced 4 - 9 egg sacs (32 - 78 eggs per sac) and 128 - 513 nauplii per female (Gaudy & Guerin, 1977). The reproductive output of *T. battagliai* measured at Brixham is within the range of values reported above for other species of *Tisbe*, however, such comparisons must be made with caution.

Differences may be explained by a number of factors including the quantity and quality of the food source, previous culture history and genetic differences between populations. It is not known whether the reproductive values observed in this study are optimal, and nutritional deficiencies in the unialgal diet and/or inbreeding depression in the laboratory cultures are potential sources of reductions in reproductive parameters.

Female *Tisbe battagliai* were capable of fertilising up to 10 broods of offspring from a single copulation. Literature values for the number of broods produced from a single copulation varied from 4 - 12 for other species of *Tisbe* and from 3 - 21 for other species of harpacticoids (Hicks & Coull, 1983). For *T. battagliai*, brood size remained relatively constant throughout the reproductive period except for the last 2 or 3 broods which tended to be smaller. Similar results were observed for the harpacticoid *Scottolana canadensis* (Harris, 1977), but other species of harpacticoids show a decrease in brood size with each successive brood (Hicks & Coull, 1983, Onbe & Kimoto, 1985).

In the current experiments, the lifespan and reproductive parameters of individual *Tisbe battagliai* showed wide variation, particularly at suboptimal food levels. These differences may be attributed to inherent biological variability which is determined partly by genotypic differences and the result of genotype versus environmental interactions. Depledge (1990, 1994) argued that the success of each species is determined by the attributes of individuals constituting the population and some individuals will be better adapted than others to respond to environmental changes. In natural populations, this biological variability has a fundamental role in the capacity of populations to respond to prevailing environmental conditions. Genetically-based differentiation in growth rate and reproductive traits were observed among latitudinally-separated populations of the harpacticoid *Scottolana canadensis* (Lonsdale & Levinton, 1986, 1989); it was hypothesised that these differences reflected local adaptation to prevailing environmental conditions (temperature) and a

possible mechanism for such adaptation was shifts in feeding efficiency to maximise available energy with changing temperature.

The field consequences of food limitation for survival and reproduction of harpacticoids is difficult to estimate due to the lack of information on the seasonal availability of food resources and its nutritional value for the different copepod life stages. Generally, laboratory investigations of harpacticoid reproduction are carried out using high quality cultured algae at constant food levels, however, these conditions may not prevail in nature. The phytal biotope offers a potentially abundant supply of food for phytal harpacticoids (Hicks, 1979), however, the seasonal availability, and associated nutritional value of specific food sources, may have different consequences for survival, development and reproduction. Limited information is available on the response of reproduction to naturally fluctuating food conditions. In laboratory studies, egg production by the harpacticoid *Scottolana canadensis* ceased following brief periods of starvation and did not recommence even when an abundant supply of food was provided later (Heinle *et al.*, 1977). The response of individual species to food limitation may reflect different life-history traits and the interpretation of results derived from laboratory experiments may be complicated by differences due to food quality. In the laboratory, adult female *Tisbe battagliai* were able to survive for relatively long periods (12 - 31 days dependent upon temperature) without algal food. Experiments were started with females derived from well-fed cultures, therefore, they may have been able to subsist for periods of time on energy reserves derived from their previous feeding regime. The test conditions did not exclude bacteria and dissolved organics, therefore, it is also likely that female *T. battagliai* were able to utilise these materials as sources of food. Female *S. canadensis* lived for many days on starvation diets and it was suggested that bacterial populations may have been sufficient to support basal metabolism (Heinle *et al.*, 1977). The harpacticoid *Nitocra spinipes* can grow and reproduce on a bacterial diet although maximal growth and development was achieved when copepods were fed a relatively lipid-rich diatom. Results from the present study suggest that

adult female *T. battagliai* have a higher tolerance to low food concentrations than the early life-history stages (Williams & Jones, 1994). Similar observations have been reported for freshwater calanoids (Jamieson & Burns, 1988).

Present results from laboratory studies on *Tisbe battagliai* suggest that reproduction may be more sensitive than growth and development to food limitation. Over the temperature range 15 - 25°C, development from hatching to the adult stage was significantly reduced at algal concentrations $\leq$ 520 μgC l^{-1} (Chapter 2; Williams & Jones, 1994) compared with significant reductions in reproductive output at algal concentrations of 1300 μgC l^{-1}. It is not known whether these differences are due solely to food quantity, or provide an indication of differences in the nutritional requirements for development and reproduction. Such differences may enable survival and development to proceed over a wider range of food conditions than those that permit reproduction. Supporting evidence is limited, although similar results were obtained by Ban (1994) who reported that egg production in the calanoid *Eurytemora affinis* was more sensitive to food shortage than somatic growth. Concerning aspects of food quality, Jonasdottir and Kiorboe (1996) reported differences in the ranking of phytoplankton species for survival and egg production in *Acartia tonsa*, indicating that the nutritional requirements for egg production and basic maintenance in this species may be different.

Information on the reproductive biology of harpacticoids from field populations is limited, however, it appears that many alga-dwelling species breed continuously (Hicks, 1977). Although temperature is recognised as an important factor controlling reproduction in harpacticoids, there are other factors which regulate breeding periodicity including food resource availability, environmental stability and their effects on the evolution of particular life-history strategies (Hicks, 1979). Predation pressure and competition may also have an important role in the evolution of life-history strategies. *Tisbe* spp are associated frequently with rocky shore seaweeds (the phytal biotope) which are reported to provide greater habitat space, a diverse and unlimited supply of food, and a

source of refuge from predators (Hicks, 1980). Macroalgal assemblages may be transitory in nature and these environments favour certain species whose life-history capabilities enable them to expand their population densities to exploit newly created resource sites (Hicks, 1982). *Tisbe* spp are regarded as typical opportunists with the ability to respond quickly to environmental disturbances through early maturation, short generation time and high levels of reproductive output. Experimental evidence suggests that these species exhibit considerable flexibility and selectivity in foods and feeding patterns that enable them to adapt to the most available food at any one time (Hicks & Coull, 1983). These abilities would also confer competitive advantage over species of harpacticoids that lack this degree of trophic plasticity.

The reproductive traits of *Tisbe battagliai* isolated here support the general description of *Tisbe* spp as opportunists (Hicks, 1980). Under optimal conditions in the laboratory (high quantity of algal food and a temperature of 20°C), *T. battagliai* develop rapidly from hatching to the adult stage (see Williams & Jones, 1994), have a rapid generation time and produce, in quick succession, numerous broods containing large numbers of offspring. Evidence from current laboratory studies suggests that temperature and food availability will have strong influences on reproductive parameters in field populations, however, the extrapolation of laboratory results to the field situation must be undertaken with caution. The factors controlling species composition and abundance of harpacticoids in the phytal biotope are complex, and the precise way in which temperature and/or food supply conspire to influence in situ reproductive periodicity with (via recruitment) its attendant effects on species abundance remains to be fully and critically evaluated (Hicks, 1985).

Table 3.1. The interaction of temperature (15, 20 and 25°C) and food (algal concentration) on female lifespan and reproductive parameters of *Tisbe battagliai*. Data were analysed by 2-way ANOVA, using a 3 (temperature) x 6 (algal concentrations of 0, 83, 208, 520, 1300 and 3250 µgC l^{-1}) factorial design for lifespan and a 3 (temperature) x 3 (algal concentrations of 520, 1300 and 3250 µgC l^{-1}) factorial design for the analysis of reproductive parameters (* significant at the 5% level; ** significant at the 1% level).

Source of variation	d.f.	Sum of squares	Mean square	F-ratio	Significance level
LIFESPAN					
Temperature	2	11716	5857.9	75.9	**
Algae	5	7467.3	1493.5	19.4	**
T x A	10	3674.3	367.4	4.8	**
Error	153	11809	77.2		
Total	179	35184			
TOTAL NUMBER OF BROODS					
Temperature	2	38.4	19.2	5.21	**
Algae	2	259	129.5	35.1	**
T x A	4	38.6	9.6	2.61	*
Error	72	265.6	3.7		
Total	89	627.7			
TOTAL NUMBER OF OFFSPRING					
Temperature	2	24021	12011	9.14	**
Algae	2	399510	199755	151.97	**
T x A	4	32260	8064.9	6.14	**
Error	72	94639	1314.4		
Total	89	560230			
NUMBER OF OFFSPRING	2	144.3	72.1	28.56	**
PER FEMALE PER	2	981.6	490.8	194.37	**
REPRODUCTIVE DAY	4	121.6	30.4	12.04	**
	72	181.8	2.53		
	89	1449.6			

Table 3.2. The effect of temperature and food (algal) concentration on the lifespan of *Tisbe battagliai*. Statistical analysis of the effect of algal concentration on female lifespan was made by making comparisons against the highest algal concentration tested (3250 μgC l^{-1}) using 1-way ANOVA followed by *t*-tests (two-tailed), or non-parametric techniques (Steel's Many-one Rank Test) to identify significant differences between the treatment means (n.s., not significant; * significant at the 5% level). Note that the values for lifespan do not include the times to first egg sac which were 16, 14 and 12 d at 15, 20 and 25°C respectively (Section 3.2.2).

Temp. (°C)	Algal conc. (μgC l^{-1})	Lifespan (days)			
		Mean	(± 1SD)	Range	Significance level
15	0	31	(4.1)	23 - 36	*
	83	27	(4.8)	20 - 33	*
	208	29	(9.1)	18 - 52	*
	520	43	(15)	16 - 56	n.s.
	1300	36	(15)	13 - 66	*
	3250	53	(9.4)	40 - 67	
20	0	14	(2)	11 - 17	*
	83	15	(7.6)	09 - 36	*
	208	36	(15)	16 - 59	n.s.
	520	35	(14)	15 - 56	n.s.
	1300	25	(7)	19 - 40	n.s.
	3250	31	(6.5)	20 - 39	
25	0	12	(5.3)	07 - 25	*
	83	12	(3.7)	07 - 21	*
	208	14	(4.2)	08 - 22	*
	520	17	(5.1)	11 - 29	*
	1300	21	(4.3)	16 - 31	n.s.
	3250	23	(4.9)	17 - 31	

Table 3.3. The effect of temperature on the lifespan of female *Tisbe battagliai*. Pairwise comparisons were made as shown and the data were statistically analysed using 1-way ANOVA followed by *t*-tests (two-tailed), or non-parametric techniques (Steel's Many-one Rank Test) to identify significant differences between the treatment means (n.s., not significant; * significant at the 5% level).

Algal concentration ($\mu gC\ l^{-1}$)	Ratio of effects on female lifespan		
	15/20°C	20/25°C	15/25°C
0	2.2 *	1.2 n.s.	2.6 *
83	1.8 *	1.3 n.s.	2.3 *
208	0.8 n.s.	2.6 *	2.1 *
520	1.2 n.s.	2.0 *	2.5 *
1300	1.4 *	1.2 n.s.	1.7 *
3250	1.7 *	1.4 *	2.3 *

Table 3.4. The effect of temperature (15, 20 and 25°C) and food concentration (algal concentrations of 0, 83, 208, 1300 and 3250 μgC l^{-1}) on the reproductive parameters of *Tisbe battagliai* [[a] denotes the number of copepods (out of 10) that produced more than one brood of offspring - the remaining copepods only produced their first brood of offspring and these were excluded from the subsequent analysis of reproductive parameters; [b] the number of female reproductive days was calculated as the total number of days between hatching of the first and last broods of offspring; [c] excluding the first brood of offspring].

Temp. (°C)	Algal conc. (μgC l^{-1})	n[a]	Total no. of female[b] reproductive days			Total no. of broods[c] produced			Total no. of offspring produced			Offspring per female per reproductive day		
			Mean	(±1SD)	Range	Mean	(±1SD)	Range	Mean	(±1SD)	Range	Mean	(±1SD)	Range
15	0	9	9.1	(0.9)	7-10	1.1	(0.3)	1-2	15	(6.9)	3-24	1.7	(0.8)	0.6-2.7
	83	6	9.8	(2.5)	7-14	1.0	(0)	1	8.3	(5.7)	2-18	1.0	(0.9)	0.1-2.6
	208	4	9.0	(2.9)	6-13	1.0	(0)	1	10	(11)	2-26	1.5	(1.9)	0.2-4.3
	520	9	27	(18)	7-46	1.7	(1.3)	1-5	13	(12)	4-45	0.8	(0.8)	0.1-2.6
	1300	8	28	(15)	10-54	3.0	(2.0)	1-6	32	(20)	4-61	1.0	(0.5)	0.4-1.6
	3250	10	29	(7.5)	18-39	5.6	(1.2)	4-7	168	(51)	78-231	5.7	(1.2)	3.7-7.5
20	0	2	3.5	(0.7)	3-4	1.0	(0)	1	14	(11)	6-22	4.4	(4.1)	1.5-7.3
	83	4	9.8	(12)	3-28	1.5	(1.0)	1-3	23	(7.8)	13-32	4.4	(2.7)	0.1-7.3
	208	8	30	(14)	6-52	3.6	(2.2)	1-8	29	(21)	3-67	0.9	(0.4)	0.4-1.3
	520	7	32	(15)	5-51	5.6	(3.1)	1-9	45	(25)	18-78	1.6	(0.9)	0.8-3.6
	1300	10	16	(8.7)	10-37	3.7	(1.8)	2-7	54	(25)	15-103	3.5	(1.1)	1.3-5.4
	3250	10	22	(5.2)	13-28	6.7	(1.7)	5-9	217	(42)	170-281	10	(2.0)	7.7-14
25	0	9	3.7	(1.0)	2-5	1.0	(0)	1	9.0	(5.3)	2-17	2.8	(1.9)	0.4-5.3
	83	7	3.7	(0.8)	3-5	1.0	(0)	1	9.1	(6.4)	5-23	2.7	(2.3)	1.0-7.7
	208	4	4.8	(1.0)	4-6	1.0	(0)	1	5.5	(3.3)	1-8	1.2	(0.7)	0.2-2.0
	520	1	17	(0)	17	3.0	(0)	3	9.0	(0)	9	0.5	(0)	0.5
	1300	10	13	(6.0)	7-26	4.2	(1.6)	3-8	68	(18)	50-113	5.9	(2.0)	4.1-8.9
	3250	10	13	(6.4)	4-25	5.3	(2.2)	2-8	125	(69)	34-232	9.6	(2.9)	3.7-14

Table 3.5. The effect of temperature on the reproductive parameters of *Tisbe battagliai*. Pairwise comparisons were made as shown and the data were statistically analysed using 1-way ANOVA followed by *t*-tests (two-tailed), or non-parametric techniques (Steel's Many-one Rank Test or Wilcoxon's Rank Sum Test) to identify significant differences between the treatment means (n.s., not significant; * significant at the 5% level).

Reproductive parameter	Algal conc. ($\mu gC\ l^{-1}$)	Ratio of effects on reproductive parameters		
		20/15°C	25/20°C	25/15°C
Total number of broods	520	3.3 *	0.5 *	1.8 *
	1300	1.2 n.s.	1.1 n.s.	1.4 n.s.
	3250	1.2 n.s.	0.8 n.s.	0.9 n.s.
Total number of offspring	520	3.5 *	0.2 *	0.7 *
	1300	1.7 *	1.3 *	2.1 *
	3250	1.3 n.s.	0.6 *	0.7 n.s.
Number of offspring per female per reproductive day	520	2.0 *	0.3 *	0.6 *
	1300	3.5 *	1.7 *	5.9 *
	3250	1.8 *	1.0 n.s.	1.7 *

Table 3.6. The number of offspring per brood for *Tisbe battagliai* reared at temperatures of 15, 20 and 25°C and algal concentrations of 520, 1300 and 3250 µgC l^{-1}. All values are means ±1SD. Note that the production of the first brood was influenced by the previous culture feeding regime.

Temp. (°C)	Algal conc. (µgC l^{-1})	Brood number									
		1	2	3	4	5	6	7	8	9	10
15	520	35 ±8.8 (n=10)	8.5 ±5.6 (n=10)	4.0 ±2.6 (n=3)	9.0 ±0 (n=1)	15 ±0 (n=1)	-	-	-	-	-
	1300	38 ±10 (n=10)	15 ±6.9 (n=8)	7.8 ±4.5 (n=6)	9.5 ±6.6 (n=4)	11 ±2.1 (n=2)	9.5 ±2.1 (n=2)	5.5 ±3.5 (n=2)	-	-	-
	3250	38 ±4.8 (n=10)	25 ±8.1 (n=10)	51 ±6.7 (n=10)	32 ±14 (n=10)	28 ±15 (n=10)	17 ±8.1 (n=8)	25 ±7.1 (n=5)	19 ±11 (n=3)	-	-
20	520	21 ±3.9 (n=9)	11 ±6.0 (n=7)	4.0 ±3.4 (n=6)	6.2 ±4.4 (n=6)	6.6 ±4.5 (n=5)	15 ±4.8 (n=4)	9.3 ±5.0 (n=4)	7.3 ±4.5 (n=3)	3.5 ±2.1 (n=2)	11 ±1.4 (n=2)
	1300	23 ±4.1 (n=10)	19 ±6.4 (n=10)	13 ±4.2 (n=10)	13 ±7.1 (n=8)	10 ±0.6 (n=3)	15 ±7.8 (n=2)	18 ±13 (n=2)	8.5 ±2.1 (n=2)	-	-
	3250	24 ±6.4 (n=10)	30 ±4.6 (n=10)	41 ±5.5 (n=10)	39 ±4.1 (n=10)	40 ±5.1 (n=10)	31 ±8.1 (n=10)	21 ±11 (n=7)	18 ±4.9 (n=4)	30 ±2.6 (n=3)	16 ±8 (n=3)
25	520	15 ±3.6 (n=10)	5.0 ±0 (n=1)	3.0 ±0 (n=1)	1.0 ±0 (n=1)	-	-	-	-	-	-
	1300	19 ±6.3 (n=10)	22 ±5.5 (n=10)	22 ±3.9 (n=10)	16 ±6.8 (n=10)	6.2 ±1.2 (n=6)	5.3 ±4.5 (n=3)	11 ±0 (n=1)	4.0 ±0 (n=1)	5.0 ±0 (n=1)	-
	3250	17 ±6.8 (n=10)	24 ±6.9 (n=10)	29 ±13 (n=10)	29 ±10 (n=9)	27 ±11 (n=8)	18 ±13 (n=7)	20 ±7.5 (n=4)	11 ±8.5 (n=4)	9.0 ±0 (n=2)	-

Table 3.7. The effect of temperature (15, 20 and 25°C) and food concentration (algal concentrations of 520, 1300 and 3250 μgC l^{-1}) on the time interval between hatching of successive broods of offspring produced by *Tisbe battagliai*.

Algal conc. (μgC l^{-1})	Brood interval	15°C				20°C				25°C			
		Mean	±1SD	Range	n	Mean	±1SD	Range	n	Mean	±1SD	Range	n
3250	1-2	6.7	±0.7	5-7	(10)	2.8	±0.6	2-4	(10)	2.4	±1.0	2-5	(10)
	2-3	3.8	±0.6	3-5	(10)	2.6	±0.5	2-3	(10)	2.0	±0	2	(10)
	3-4	4.4	±0.7	4-6	(10)	2.8	±0.4	2-3	(10)	2.1	±0.3	2-3	(9)
	4-5	5.5	±3.1	4-14	(10)	2.6	±0.5	2-3	(10)	2.1	±0.4	2-3	(8)
	5-6	6.8	±2.1	4-11	(8)	3.8	±2.2	3-10	(10)	3.0	±1.2	2-5	(7)
	6-7	4.6	±1.1	3-6	(5)	4.3	±2.6	3-10	(7)	2.3	±0.5	2-3	(4)
	7-8	4.3	±0.6	4-5	(3)	5.0	±2.0	4-8	(4)	3.8	±1.0	3-5	(4)
	8-9	-	-	-	-	2.3	±0.6	2-3	(3)	5.5	±3.5	3-8	(2)
	9-10	-	-	-	-	3.3	±0.6	3-4	(3)	-	-	-	-
1300	1-2	11	±5.0	5-22	(8)	3.8	±1.0	3-6	(10)	2.2	±0.4	2-3	(10)
	2-3	11	±4.2	6-17	(6)	5.3	±0.7	4-6	(10)	2.4	±0.5	2-3	(10)
	3-4	8.3	±4.0	6-14	(4)	4.3	±1.6	3-8	(8)	3.0	±0.5	2-4	(10)
	4-5	6.0	±1.4	5-7	(2)	6.3	±4.0	4-11	(3)	4.7	±1.6	3-7	(6)
	5-6	6.0	±1.4	5-7	(2)	4.0	±2.8	2-6	(2)	4.3	±1.5	3-6	(3)
	6-7	6.0	±1.4	5-7	(2)	4.5	±2.1	3-6	(2)	4.0	±0	4	(1)
	7-8	-	-	-	-	4.0	±1.4	3-5	(2)	3.0	±0	3	(1)
	8-9	-	-	-	-	-	-	-	-	3.0	±0	3	(1)
	9-10	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.7. continued. The effect of temperature (15, 20 and 25°C) and food concentration (algal concentrations of 520, 1300 and 3250 µgC l^{-1}) on the time interval between hatching of successive broods of offspring produced by *Tisbe battagliai*.

Algal conc. (µgC l^{-1})	Brood interval	15°C				20°C				25°C			
		Mean	±1SD	Range	n	Mean	±1SD	Range	n	Mean	±1SD	Range	n
520	1-2	16	±15	7-44	(9)	7.0	±4.3	3-15	(7)	4.0	±0	4	(1)
	2-3	27	±6.0	21-33	(3)	7.5	±3.8	4-14	(6)	5.0	±0	5	(1)
	3-4	2.0	±0	2	(1)	6.6	±5.4	3-16	(5)	8.0	±0	8	(1)
	4-5	5.0	±0	5	(1)	5.0	±2.5	3-9	(5)	-	-	-	-
	5-6	6.0	±0	6	(1)	3.3	±0.5	3-4	(4)	-	-	-	-
	6-7	-	-	-	-	3.5	±0.6	3-4	(4)	-	-	-	-
	7-8	-	-	-	-	4.7	±0.6	4-5	(3)	-	-	-	-
	8-9	-	-	-	-	8.5	±4.9	5-12	(2)	-	-	-	-
	9-10	-	-	-	-	7.0	±2.8	5-9	(2)	-	-	-	-

Table 3.8. The effect of temperature and food (algal) concentration on the reproductive parameters of *Tisbe battagliai*. Statistical analysis of the effect of algal concentration on reproductive parameters was made by making comparisons against the highest algal concentration tested (3250 μgC l^{-1}) using 1-way ANOVA followed by *t*-tests (two-tailed), or non-parametric techniques (Steel's Many-one Rank Test or Wilcoxon's Rank Sum Test) to identify significant differences between the treatment means (n.s., not significant; * significant at the 5% level).

Reproductive parameter	Algal conc. (μgC l^{-1})	Temperature		
		15°C	20°C	25°C
Total number of broods	520	1.7 *	5.6 n.s.	3.0 *
	1300	3.0 *	3.7 *	4.2 n.s.
	3250	5.6	6.7	5.3
Total number of offspring	520	13 *	45 *	9 *
	1300	32 *	54 *	68 n.s.
	3250	168	217	125
Number of offspring per female per reproductive day	520	0.8 *	1.6 *	0.5 *
	1300	1.0 *	3.5 *	5.9 *
	3250	5.7	10	9.6

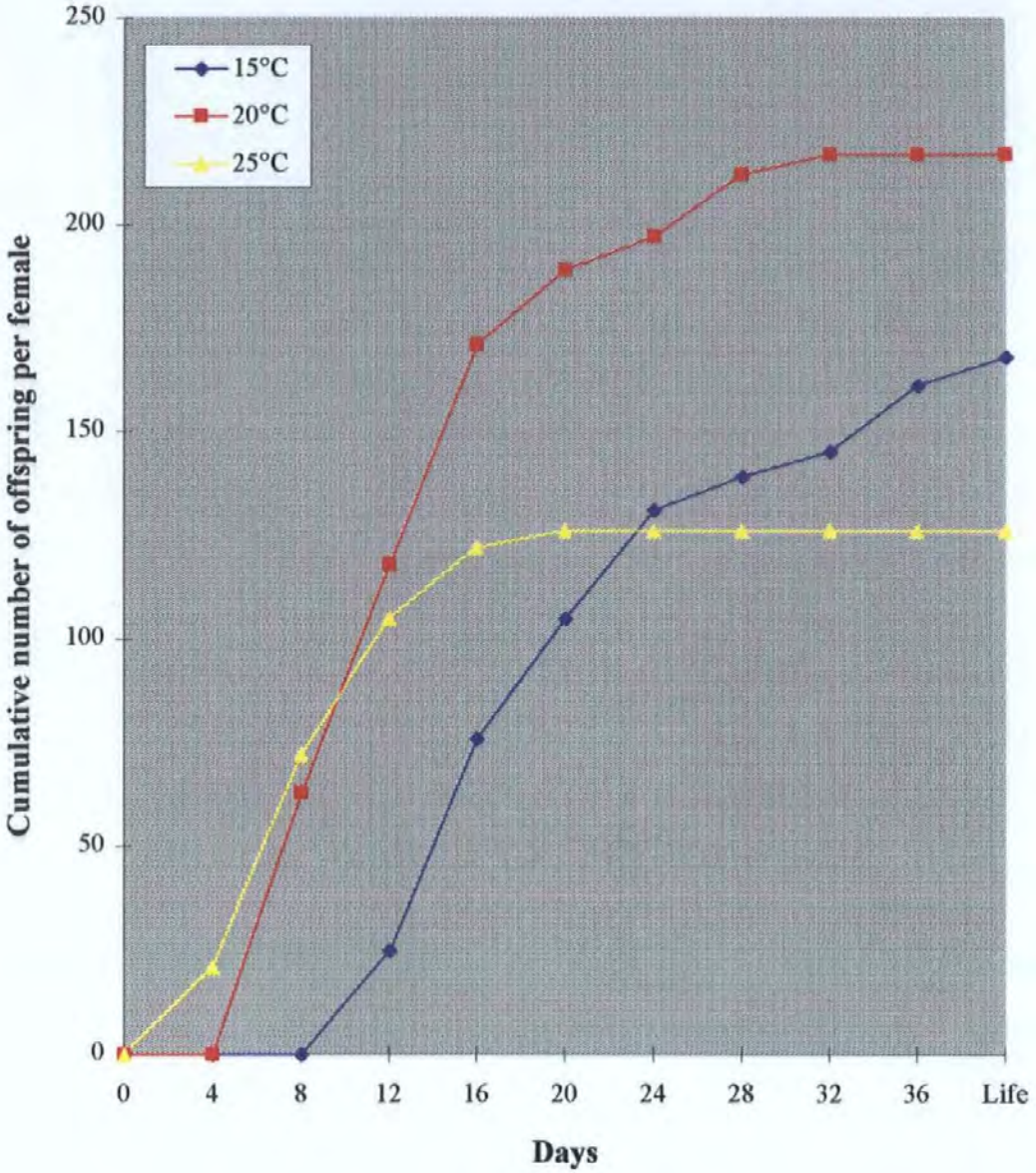


Figure 3.1. The effect of temperature (15, 20 and 25°C) on offspring production by *Tisbe battagliai* fed an algal concentration of 3250 µgC l. Results are expressed as the cumulative number of offspring produced by each female (mean of n=10) during their lifespan. Variation around the mean values (lifespan only) is shown in Table 3.4.

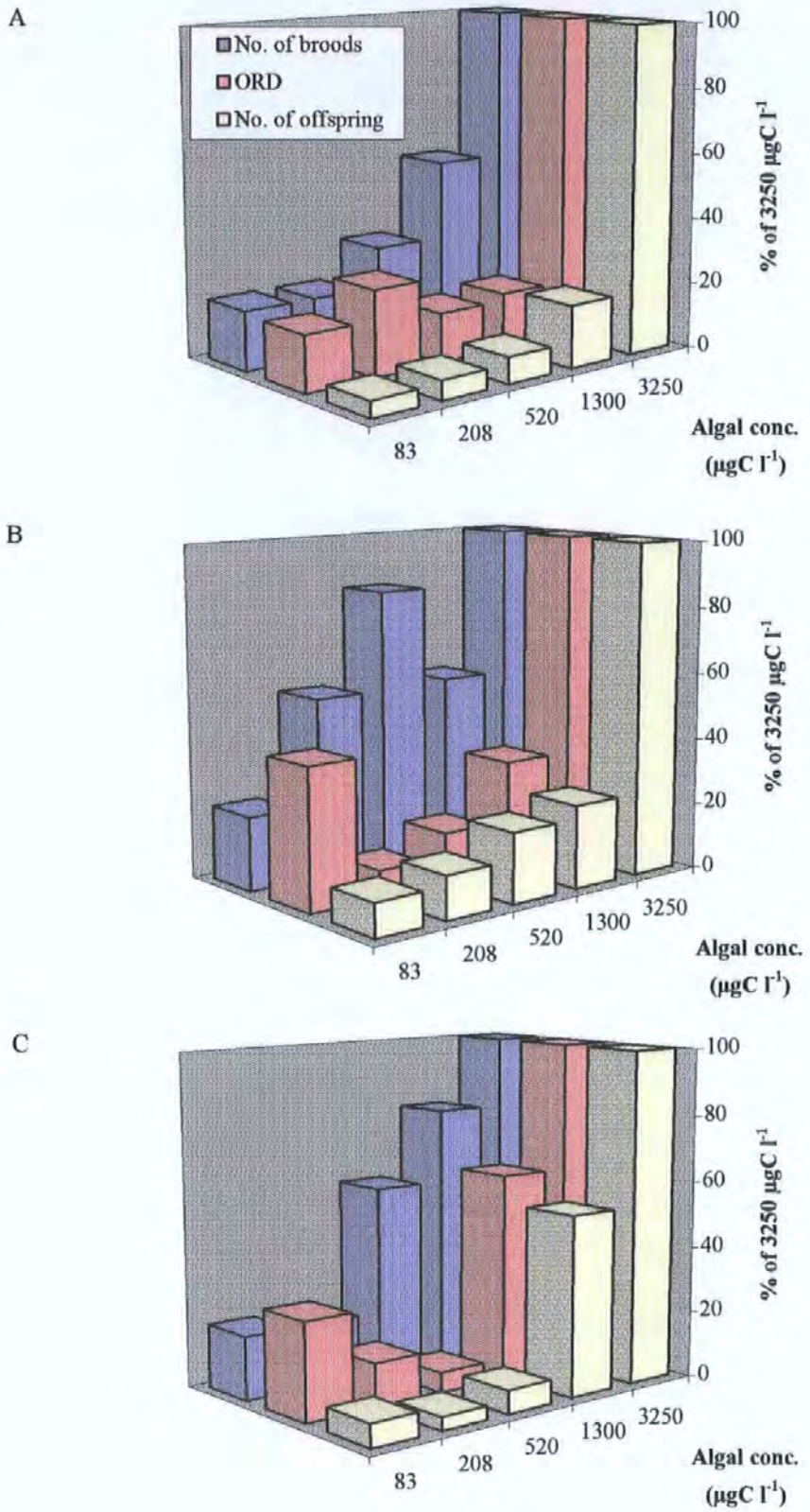


Figure 3.2. The effect of food (algal) concentration on the reproductive parameters [total number of broods, number of offspring per female per reproductive day (ORD) and total number of offspring produced] of *Tisbe battagliai* at temperatures of (A) 15°C, (B) 20°C and (C) 25°C. Effects at different algal concentrations were compared with performance at the highest algal concentration tested (3250 µgC l). Data are taken from the mean values shown in Table 3.4.

CHAPTER 4

EFFECTS OF TEMPERATURE AND FOOD QUANTITY ON THE COMPLETE LIFE CYCLE OF *TISBE BATTAGLIAI* (COPEPODA: HARPACTICOIDA)

4.1. INTRODUCTION

Temperature and food concentration had a significant influence on the postembryonic development and reproduction of *Tisbe battagliai* (Chapters 2 and 3 respectively). These biological measurements have ecological relevance only insofar as these components of the life cycle affect the primary population parameters of birth, death and migration of organisms. To examine the way in which the properties of individuals determine population dynamics, a different approach is required. The format most often used to tabulate and examine patterns of survivorship and fecundity is known as a life table. Life tables are of the utmost importance because they contain the raw material of the 'ecological fact of life' (Begon *et al.*, 1990). Life tables are also the prime source of information on age-specific characteristics and, ultimately, on reproductive strategies (Bergmans, 1983). Since their introduction by Leslie and Ranson (1940) and Birch (1948), ecologists have used life tables to assess the growth rate of populations under various environmental conditions. In cohort life-table experiments, a single cohort of organisms (i.e. a group of individuals born within the same short time interval) are followed from birth to death while studying effects on longevity, rate of development, and fecundity. One way of combining reproduction and mortality data for populations utilises a demographic parameter called the intrinsic rate of natural increase (r_m). The r_m is an ecological measure which expresses the growth potential of a population in an unlimited environment (optimum conditions where competition and predation are not influencing factors). The intrinsic rate of natural increase, which is calculated from life-table data, integrates survival, age at first reproduction, brood size and frequency, and longevity to determine net population changes. This parameter is an important component of ecological theory (Krebs, 1994) and can improve our understanding of how population processes are influenced by environmental factors.

The intrinsic rate of increase is a statistical characteristic of a population and depends upon environmental conditions (Andrewartha & Birch, 1954). In nature, environments vary continuously, therefore, when conditions are favourable, numbers increase (r_m is positive); when conditions are unfavourable they decrease (r_m is negative). In nature, the actual rate of increase varies continuously from positive to negative in response to changes within the population in age distribution and genetic composition, and in response to changes in environmental factors. In populations with overlapping generations (continuous breeding), r_m is the intrinsic rate of natural increase that the population is capable of achieving but will only be achieved if the survivorship and fecundity schedules remain steady over a long period of time. If these conditions are met, r_m will be approached (and thereafter maintained) and over the same period the population will approach a stable age structure (i.e. one in which the proportion of the population in each age class remains constant over time) which is maintained indefinitely. The concept of r_m is an oversimplification, as in nature, we do not find populations with stable age distributions or with constant age-specific rates of mortality and fecundity. The actual rate of increase in natural populations varies in more complex ways than the theoretical constant r_m. It can, however, be useful to characterise a population in terms of its potential, especially when the aim is to make a comparison (e.g. comparing populations of the same species in different environments) to see which environmental conditions appears to be the most favourable for the species.

Life-table data provide a valuable insight into the population dynamics of harpacticoids. Published studies on the demography and reproductive strategies of harpacticoid copepods have been examined critically by Bergmans (1984a). Data for several species of *Tisbe* are available (Hicks & Coull, 1983), including interspecific (Bergmans, 1981, 1984b) and intraspecific (Bergmans & Janssens, 1988) comparisons. Life-table data have also been

proposed as an ecologically relevant parameter in environmental hazard assessments (Bechmann, 1994).

The aim of the present chapter was to investigate the effect of environmental variables (temperature and food concentration) on demographic parameters (e.g. lifespan, reproductive period, minimum generation time, total production of offspring) and to establish their subsequent effect on the intrinsic rate of increase (r_m) of the marine copepod *Tisbe battagliai*.

4.2. MATERIALS AND METHODS

4.2.1. Test procedure

Experiments were carried out in temperature-controlled rooms (± 1°C) with a photoperiod of 16 h light: 8 h dark. For the life-table analyses, 50 newly released nauplii (< 7 h old) were reared at three different temperatures (15, 20 and 25°C) and six concentrations of the alga *Isochrysis galbana* (0, 83, 208, 520, 1300 and 3250 µgC l^{-1}). Cohort synchrony was achieved using nauplii released within 7 h from parturient females (n = ≈ 50) isolated from the main cultures in a crystallising dish containing 200 ml of seawater and 2 x 10^5 cells ml^{-1} *I. galbana*. The temperatures investigated in this study (15 - 25°C) covered the upper seasonal range experienced by *Tisbe battagliai* in British and Mediterranean coastal waters (Williams & Jones, 1994). The algal concentrations were selected to cover the range of 0 (no algal food) to 3250 µgC l^{-1}, an algal concentration which has previously been shown to support relatively high offspring production in the laboratory (Chapter 3). The intrinsic rate of natural increase is applied here, therefore, to life-cycle data observed in a competitor free environment with optimal resources (e.g. food quantity, space). For the purposes of this study, an algal concentration of 3250 µgC l^{-1} was considered to provide unlimited food resources for all life stages of *T. battagliai*. Algal concentrations of 1300 µgC l^{-1} (and lower) were included in the

experimental design to measure the effect of limiting food concentrations on r_m and to provide a comparison with the results from previous chapters which examined the effect of temperature and food concentration on postembryonic development and reproduction of *T. battagliai* (Chapters 2 and 3 respectively).

To limit the transfer of algal cells, nauplii were sieved carefully through a 62 μm nylon mesh and resuspended in freshly filtered seawater before being added to the experimental containers. Stock suspensions of *Isochrysis galbana* were diluted with filtered (0.2 μm) seawater to provide concentrations of 0 (no algae), 83, 208, 520, 1300 and 3250 μgC l^{-1}; each concentration increasing stepwise by a factor of 2.5. Corresponding values (mean measured), based on algal cell numbers, ranged from ≈ 0.12 - 4.6 x 10^5 cells ml^{-1} (83 - 3250 μgC l^{-1}). Carbon values, and cell concentrations for the freshly prepared algal stock suspensions, were determined using a Dohrman DC-190 high temperature carbon analyser and a Model ZB Coulter Counter, respectively. Algal stock suspensions were stored in a refrigerator and used within 7 days.

Fifty nauplii (< 7 h old) were added randomly to crystallising dishes containing 200 ml of test solution and maintained at each combination of temperature and food concentration until death. As soon as females extruded their first egg sac they were transferred individually to wells of tissue culture plates each containing 5 ml of test solution; males were retained in the original vessel. Observations of mortality and fecundity were made daily and the surviving copepods were transferred to duplicate test chambers containing freshly prepared test solutions. Transfers and observations during the experiments were carried out with a Wild M8 binocular microscope, using darkfield illumination. Temperature (daily) and the pH and dissolved oxygen concentration (at least weekly) was measured in each treatment.

4.2.2. Life-table calculations

Demographic parameters were calculated using the procedures described by Bergmans (1981, 1983, 1984ab). Fundamental quantities in any demographic analysis include measures of offspring number, temporal pattern of births, and measures incorporating both these elements (Bergmans, 1984). All these measurements may be derived from the age-specific survival (l_x) and fecundity (m_x) schedules which are used to compute the intrinsic rate of natural increase (r_m) The intrinsic rate of natural increase was calculated from life-table data by solving the equation derived by Lotka (1925):

$$\sum l_x m_x e^{-rx} = 1$$

where

l_x = number of living females on day x/number of females at the start of the life table

x = age (days)

m_x = number of female offspring (nauplii) produced on day x/number of living females on day x

$U_x = l_x m_x$ = number of female offspring (nauplii) produced on day x/number of females at the start of the life table

$R_o = \sum l_x m_x = \sum U_x$

This equation is solved by iteration and detailed descriptions of how these calculations are performed are provided by Andrewartha and Birch (1954) and Pielou (1977).

For a cohort of animals observed from birth through death at regular intervals (x), the age-specific survival (l_x) is the probability of surviving to age x and is found by dividing the number

of living females of age x (days) by the number of females at the start of the life table. Because survival of the females is most important to reproduction, only the age-specific survival of the females has been calculated in the life tables. Sexual differentiation in *Tisbe* is not evident until at least the fourth copepodid stage (Bergmans, 1981), therefore, the numbers of living females at the start of the experiments, and during development to the adult stage, were estimated using the observed proportion of females recorded after sex determination was made (including all sexed animals, alive and dead). In experiments where no mortalities occurred prior to sex determination, the number of females was recorded directly. It was assumed that the mortality of males and females was the same during the first part of the experiments. Age-specific fecundity (m_x) is the number of female offspring produced on day x per living female. When calculating the age-specific fecundity, it was assumed that, regardless of the temperature and food concentration, 50% of the offspring were females (i.e. a 1:1 sex ratio). The realised fecundity (U_x) is the number of new born females produced per day per new born female of the preceding generation. U_x summarised over the entire test period gives the net reproductive rate (R_o), the factor by which the female generation is multiplied from one generation to the next. If the net reproductive rate is 1.0, the population is replacing itself exactly. When the net reproductive rate is below 1.0, the population is not replacing itself. In addition to r_m, the following life-history parameters were also quantified: lifespan (from birth to age at death) of males and females, and for fertile females; reproductive period, time to first reproduction, total number of broods, total number of offspring and the number of offspring per female per reproductive day.

4.2.3. Statistical analysis

The effects of temperature and food concentration on lifespan, reproductive period, time to first reproduction, number of broods, number of offspring and number of offspring per

female per reproductive day were determined using 1-way ANOVA followed by Student's *t*-tests (two-tailed) to identify significant differences between the treatment means. Data that failed to meet the assumptions for analysis of variance were analysed using Wilcoxon's Rank Sum Test, a non-parametric technique (Hollander & Wolfe, 1973). The sex ratio of adult copepods was examined using a contingency table procedure (Hayslett & Murphy, 1976) and comparisons between the effect of temperature on male and female lifespan by Student's *t*-test (two-sided).

4.3. RESULTS

4.3.1. Effect of temperature on r_m (unlimited food concentration)

The demographic data for *Tisbe battagliai* reared at 15, 20 and 25°C in unlimited food concentrations (3250 μgC l^{-1}) are shown in Table 4.1. These data were calculated from daily observations of survivorship and fecundity of *T. battagliai* nauplii from hatching through to day at death. Juvenile survival rates were high and 80 - 94% of nauplii in each cohort successfully reached the adult stage. The sex ratio of adult copepods did not depart significantly from 1:1 (Table 4.1) although the proportion of males was higher at 20°C and 25°C (68 - 70%) than it was at 15°C (40%). In each cohort, 72-92% of females were fertile and produced viable egg sacs. Lifespan of male and females copepods was inversely related to temperature and both sexes lived, on average, approximately 3 times longer at 15°C than at 25°C (Table 4.1). Infertile females did not live as long as reproductive females, however, no statistical comparisons were made due to the relatively small number of infertile copepods involved (Table 4.1). Lifespan of fertile females was significantly longer (Student's *t* - test, $P < 0.05$) than males at corresponding test temperatures and these differences amounted to ≈ 24 days at 15°C, 13 days at 20°C and 3 days at 25°C.

Values of r_m at 20 and 25°C (0.182 and 0.177 d^{-1} respectively) were higher (44-46%) than r_m at 15°C (0.099 d^{-1}). At the lower temperature, a significant decrease in the rate of development and fecundity contributed to a reduction in r_m (Table 4.1). Development proceeded faster at higher temperatures as illustrated by the day of first brood release (28 days at 15°C, 16.9 days at 20°C and 12.1 days at 25°C). Significant decreases in time to first reproduction with increasing temperature were followed by significant reductions in female lifespan and reproductive period (Table 4.2). Differences were most pronounced between the extremes of the temperature range (15 and 25°C). For example, the mean lifespan of egg producing females was reduced from 83.2 to 25.3 days and reproductive period from 39.5 to 6.82 days at the temperature extremes.

At 25°C, copepods produced significantly fewer broods and significantly less numbers of offspring than those reared at 15 and 20°C (Table 4.2). These results may be explained by the significantly shorter lifespan and reproductive period at the higher temperature. By comparison, the rate of reproduction, expressed as the number of offspring per female per reproductive day, was significantly lower at 15°C (3.95 nauplii per day) than at 20 and 25°C (7.44 and 9.29 nauplii/day respectively); differences were not significant at 20 and 25°C (Table 4.2). The effects of increasing temperature on the lifespan and reproduction of *Tisbe battagliai* are illustrated clearly by the age-specific survival and fecundity schedules (Fig. 4.1); reproduction begins earlier, but female longevity and the duration of reproduction is shorter, with increasing temperature.

4.3.2. Effect of food concentration on r_m

Development from the nauplius to the adult stage was sensitive to relatively small changes in algal concentration and only 44 - 52% of each cohort reached adulthood following a reduction in algal concentration from 3250 to 1300 µgC l^{-1} (2.5 times less algae). With

further reductions in food concentration, fewer numbers of copepods successfully reached the adult stage and the effects were more pronounced with increases in temperature (Fig. 4.2). Copepod survival and development in low food concentrations (0 - 208 μgC l^{-1}) was very poor, therefore, only the life-table data for algal concentrations of 520, 1300 and 3250 μgC l^{-1} are shown (Tables 4.3 - 4.5). In these life tables, there is no clear trend in male or female lifespan due to algal concentration, however, lifespan is only shown for those copepods that reached the adult stage. Taking all members of the original cohort population (n = 50), there is a general trend of a reduction in lifespan with successive reductions in algal concentration (Fig. 4.3). With successive reductions in food concentration below 3250 μgC l^{-1}, r_m moved gradually from positive to negative values (Table 4.6).

At 15°C, values of r_m remained positive despite a relatively large reduction at 1300 μgC l^{-1} (42%) and 520 μgC l^{-1} (68%). At these algal concentrations, reductions in r_m were attributed to significant reductions in the time to first reproduction and fecundity (Table 4.3). At lower algal concentrations (83 and 208 μgC l^{-1}), only one female in each concentration produced viable offspring and values of r_m became negative. Only 6 - 10% of copepods reached the adult stage at 83 and 208 μgC l^{-1} and, because of the small numbers, it was not possible to determine accurately the adult sex ratio. Consequently, a 1:1 sex ratio was assumed for the life-table analysis, however, effects on r_m may have been underestimated if food limitation had caused changes in the copepod sex ratio (see Discussion).

At 20°C, reductions in algal concentration below 3250 μgC l^{-1} resulted in a decrease (58%) in r_m at 1300 μgC l^{-1} and a decrease (74%) at 520 μgC l^{-1} although r_m values remained positive (Table 4.4). No copepods survived to reproductive maturity at 83 and 208 μgC l^{-1} and these populations died out.

At 25°C, copepods fed algal concentrations lower than 1300 μgC l^{-1} died before reaching adulthood (Table 4.5). At 1300 μgC l^{-1}, 44% of the cohort survived to the adult

stage although the sex ratio was significantly biased towards males (18 males and 4 females); only 2 of these females managed to produce egg sacs but no offspring were hatched and the population died out (Table 4.5).

4.4. DISCUSSION

Any evaluation of population processes in harpacticoids should be based on sound demographic theory. Published studies on the demography and reproductive strategies of harpacticoid copepods were examined critically by Bergmans (1984) who reported confused and biased estimates of certain demographic parameters. For example, Bergmans (1984) considered that the popular approximation of r_m (In R_o/T_c) was an inappropriate estimate of the intrinsic rate of natural increase because it leads to a systematic underestimation (by 8 - 29%) for life histories typical of fast breeding species. In view of these concerns, interpretation of published life-table data should be undertaken with care.

Compared with its sibling species *Tisbe holothuriae*, the life history of *T. battagliai* is relatively unknown. Examination of the published literature (Bergmans, 1981; Hicks & Coull, 1983) reveals information on population parameters for several species within the genus *Tisbe* but only one other study reports demographic variables for *T. battagliai* (Bergmans & Janssens, 1988). Comparison between different studies is difficult due to the different temperature and salinity regimes used. However, when the single most important fitness measures in stationary and expanding populations are considered (i.e. R_o and r_m respectively), the performance of *T. battagliai* reared at 20°C and 35‰ (this study) and those reared at 18°C and 20 & 32‰ (Bergmans & Janssens, 1988) are similar. At these temperatures and salinities, values of R_o for *T. battagliai* range from 58.6 - 61.6, r_m from 0.188 - 0.203 d^{-1} and T_{min} from 16.2 - 18.2 (Table 4.7). These values are lower than the corresponding demographic

parameters for *T. holothuriae* reared at similar temperature and salinity regimes (18°C and 33 - 36‰) (Table 4.7). Demographic parameters for various laboratory strains of *T. holothuriae* ranged from 86 - 187 for R_o, 0.27 - 0.32 for r_m and 11.2 - 11.9 for T_{min} (Bergmans, 1981). More recent data for *T. holothuriae* provided values of 75.3 for R_o, 0.263 for r_m and 13.7 for T_{min} (Bergmans & Janssens, 1988). The latter are just outside the lower range of values reported above, however, Bergmans & Janssens (1988) noted that their laboratory diet (lettuce) may not have been optimal. Lower fecundity, and an increase in generation time (T_{min}), appears to be the main cause of differences in demographic parameters between *T. battagliai* and *T. holothuriae* but further data are required to establish whether these differences between the sibling species are due to differences in life-history attributes or to an artefact of laboratory experiments. Examination of fitness measures (R_o and r_m) between these two species has revealed that *T. battagliai* is little affected by salinity differences of 20 and 35‰, whereas performance of *T. holothuriae* is greatly reduced at 20‰ (Bergmans & Janssens, 1988). Other factors (e.g. nutritional factors) may contribute to the observed differences in demographic data between these species.

Intrinsic factors known to affect r_m are principally fecundity, longevity and rate of development (e.g. Bergmans, 1981); extrinsic factors are mainly temperature, salinity and nutrition (e.g. Hicks & Coull, 1983 and references contained therein). Copepods were fed a unialgal diet of *Isochrysis galbana* and a concentration of 3250 $\mu gC\ l^{-1}$ has, from previous studies, been shown to support high levels of survival, development and reproduction (Chapters 2 and 3). This algal concentration was judged to provide unlimited food resources in life-table experiments yet it is not known whether this diet is optimal. Usually, reproductive output increases at optimal, as opposed to suboptimal, food regimes, therefore, the importance of food quality, and its potential effect on population parameters, should not be ignored. The importance of food quality in copepod diets has been discussed previously (Chapters 2 and 3)

and several authors have noted the benefits of mixed algal diets for offspring production in harpacticoids (Nassogne, 1970; Harris, 1977). The results from laboratory experiments indicate that reproduction is sensitive to relatively small reductions in food concentration but in the field situation, where a greater diversity of foods is usually available, responses may be influenced by the type of diet (i.e. food quality). Further work is required to establish the effect of food quality on the population dynamics of *Tisbe battagliai* and a mixed species algal diet was investigated as a potential source of a more nutritionally complete diet (Chapter 5).

The life-table data for individual females compare favourably with the results from previous experiments to determine the effect of temperature and food concentration on reproduction of *Tisbe battagliai* (Chapter 3). In Chapter 3, reductions in algal concentrations from 3250 to 1300 µgC l^{-1} resulted in a significant decrease in offspring production and these results are in general agreement with those from cohort life-table experiments (this Chapter). In unlimited food concentrations (3250 µgC l^{-1}), the mean values for lifespan and reproductive output (number of broods, number of offspring, number of offspring per female per reproductive day) reported for individual females in reproduction (Chapter 3) and life-table experiments (this Chapter) were within a range of 1.04 - 1.45 (15 and 20°C) and 1.03 - 2.8 (25°C).

Survival and reproductive output varied greatly between individuals in a cohort of *Tisbe battagliai* and this variability may be a feature of natural populations. Differences between individuals are the raw materials for evolutionary change and for the evolution of adaptations (Bennett, 1987), therefore, describing the functional response in terms of the average organism in the group may ignore important attributes of individuals in the population. Wide variations in survival and reproductive output of individual *T. battagliai* were found in previous experiments (Chapter 3) and the implications of these findings are discussed more

fully therein. Bechmann (1994) reported similar variability in the survival and reproduction of individuals in a cohort of *T. furcata*.

As a result of predation and competition, survival rates in the field are often much lower than those observed in the laboratory (Gehrs & Robertson, 1975; D'Apolito & Stancyk, 1979). As these biotic factors are usually excluded from laboratory experiments, r_m does not necessarily describe the rate of population growth in the field but is a useful way of comparing other laboratory studies. Calculation of r_m from survival and fecundity schedules assumes a sex ratio of 1:1, however, data have shown that the sex ratios of harpacticoids may vary considerably in the laboratory and in the field (Hicks & Coull, 1983). Laboratory culture conditions may also influence the demography of *Tisbe* spp. For example, genetic impoverishment of the laboratory culture may result in inbreeding depression which reduces fecundity (Lazzaretto & Parise, 1967; Lazzaretto-Colombera *et al.*, 1976; Fava *et al.*, 1976) and changes sex ratio (Battaglia, 1958). In the laboratory, populations are more likely to be descended from a common ancestor due to relatively small effective population size, while in natural populations the reduction of variability can be a much slower process, under the direction of natural selection (Fava & Martini, 1988). The genus *Tisbe* appears to be extremely sensitive to the effects of protracted inbreeding in both field and laboratory populations, and many species investigated in the laboratory show male dominance (Bergmans, 1981; Hicks & Coull, 1983). In *Tisbe*, sex determination is known to be genetic (Battaglia, 1961; Battaglia & Malesani, 1962; Scudo, 1967) and sensitive both to the degree of inbreeding (Battaglia, 1958; Lazzaretto-Colombera *et al.*, 1976) and the time of fertilisation (Volkmann-Rocco, 1972). Modification of sex in harpacticoids has been demonstrated to be under the influence of a range of environmental and biotic variables. Amongst those variables suggested to influence sex ratio in both laboratory and field populations are temperature, salinity, food supply (both quantity and quality) and population density or crowding (Hicks & Coull, 1983

and references contained therein). In field data, inverse correlations have been noticed regularly between density and dominance by females, and this has been predicted to be an intrinsically self-regulating phenomenon (Hicks, 1977). Predominance of females at low population density ensures there are sufficient females in reproductive condition; an adaptation for survival at low densities (Hicks, 1977). Even at low densities, there are usually sufficient numbers of males present that are capable of inseminating multiple females; spermatophore-mediated sperm transmission also enables females to fertilise successive broods from a single insemination. In the present study, it is not known whether the high incidence of 'maleness' observed in cohorts reared at 20 and 25°C is due to inbreeding depression or to the other factors noted above. Additional data for *Tisbe battagliai* are required to confirm sex ratios under laboratory conditions. While there is no reason to believe that the genetic sex ratio of harpacticoids should depart from 1:1 (Hicks & Coull, 1983), the phenotypic expression of sex in the *Tisbe* genus almost always deviates from equality. Consequently, changes in sex ratio should be borne in mind when interpreting the results of life-table analysis (which assumes a sex ratio of 1:1 for the offspring produced) for species of *Tisbe*.

Changes in the life-history attributes of a species can affect the intrinsic rate of natural increase. In general, three factors have been shown to increase r_m: reduction in age at first reproduction, increased fecundity and increased number of reproductive bouts (increased survival or longevity). The most pronounced effects are usually achieved by changes in the age at first reproduction and the earlier the peak in reproductive output, the larger the value of r_m as a result (Krebs, 1994). Data from this study provide a good example to illustrate this principle in *Tisbe battagliai*. Copepods reared at 25°C survived poorly (female lifespan was 25.2 days) and produced fewer numbers of offspring (44.7 offspring per female) than those reared at 20°C (lifespan was 51.2 days, offspring per female was 157). However, because females began to reproduce at an earlier age at 25°C (12.1 compared with 16.9 days) and had a

shorter generation length, its r_m was very similar to the longer living, more fecund females reared at 20°C.

The results from cohort life-table experiments and those from earlier experiments (Chapters 2 and 3) confirm that development and reproductive output in *Tisbe battagliai* are strongly temperature dependant. These results were predictable and are consistent with published information on the effect of temperature on development and reproduction in marine harpacticoids (Hicks & Coull, 1983). By comparison, much less is known about the nutritional requirements of harpacticoids and how these animals respond to food resources by way of regulation of reproductive periodicity and population dynamics. The present series of experiments has established that the population dynamics of *T. battagliai* are strongly affected by food quantity but the relative importance of food quality requires further investigation.

Table 4.1. Summary of results (mean ±1 SD) from cohort life-table experiments with *Tisbe battagliai* reared in unlimiting food concentration (3250 µgC l^{-1}) at temperatures of 15, 20 and 25°C. Statistical comparison between the reproductive parameters is shown in Table 4.2.

Parameter	Temperature		
	15°C	20°C	25°C
% Copepods that reached the adult stage	84 (n=42)	94 (n=47)	80 (n=40)
% Males	40 (n=17)	68 (n=32)	70 (n=28)
% Females	60 (n=25)	32 (n=15)	30 (n=12)
% Egg-producing females	72 (n=18)	80 (n=12)	92 (n=11)
% Infertile females	28 (n=7)	20 (n=3)	08 (n=1)
Lifespan (days) of			
Males	59.6 ±24.5	41.3 ±9.96	22.8 ±7.21
Females	70.3 ±26.4	50.7 ±23.0	24.7 ±6.87
Egg-producing females	83.2 ±16.2	54.3 ±24.2	25.3 ±6.86
Infertile females	37.1 ±16.0	32.7 ±8.96	18.0 ±0
Reproductive period (days) of egg-producing females	39.5 ±14.0	21.6 ±16.5	6.82 ±7.25
No. of broods/ egg-producing females	8.00 ±2.8	6.42 ±4.32	2.82 ±2.36
No. of nauplii/ egg-producing females	156 ±82	157 ±141	44.7 ±47.2
Offspring per female per reproductive day	3.95 ±1.30	7.44 ±3.30	9.29 ±4.58
T_{min} = minimum generation time (days)	28.0 ±2.9	16.9 ±2.68	12.1 ±1.04
T_c = cohort generation time (days)	47.4	28.9	19.2
$\sum m_x$ = summation of age-specific fecundity	78.2	87.8	46.1
$R_o = \sum U_x$ = net reproductive rate per generation	49.1	59.9	16.4
r_m = intrinsic rate of natural increase (d^{-1})	0.099	0.182	0.177

Table 4.2. Results of one-way analysis of variance (followed by *t*-tests) or Wilcoxon's Rank Sum Test of the effect of temperature (15, 20 and 25°C) on reproductive parameters of egg-producing females reared in unlimiting food concentration of 3250 μgC l^{-1} (n.s., not significant; * significant at the 5% level; ** significant at the 1% level).

Parameter	Temperature (°C)	Significance level
Lifespan (days)	15 vs 20 vs 25	*
Reproductive period (days)	15 vs 20 vs 25	**
Time to first reproduction (days)	15 vs 20 vs 25	*
Number of broods	15 vs 20	n.s.
	25 vs 15	**
	25 vs 20	**
Number of offspring	15 vs 20	n.s.
	25 vs 15	*
	25 vs 20	*
Offspring per female per reproductive day	15 vs 20	*
	15 vs 25	*
	20 vs 25	n.s.

Table 4.3. Summary of results (mean ±1 SD) from cohort life-table experiments with *Tisbe battagliai* reared at 15°C and algal concentrations of 520, 1300 and 3250 µgC l^{-1} [* significant difference ($P < 0.05$) compared with highest algal concentration].

Parameter	Algal concentration (µgC l^{-1})		
	520	1300	3250
% Copepods that reached the adult stage	26 (n=13)	46 (n=23)	84 (n=42)
% Males	54 (n=7)	48 (n=11)	40 (n=17)
% Females	46 (n=6)	52 (n=12)	60 (n=25)
% Egg-producing females	17 (n=5)	83 (n=10)	72 (n=18)
% Infertile females	83 (n=1)	17 (n=2)	28 (n=7)
Lifespan (days) of			
Males	61.9 ±25.9	38.5 ±18.7	59.6 ±24.5
Females	74.5 ±10.3	83.7 ±27.4	70.3 ±26.4
Egg-producing females	74.6 ±11.5	94.1 ±13.9	83.2 ±16.2
Infertile females	74.0 ±0	31.5 ±0.71	37.1 ±16.0
Reproductive period (days) of egg-producing females	31.4 ±16.8	58.3 ±17.3	39.5 ±14.0
No. of broods/ egg-producing females	4.00 ±2.35*	7.30 ±3.40	8.00 ±2.8
No. of nauplii/ egg-producing females	53.8 ±19.5*	111 ±75.2	156 ±82
Offspring per female per reproductive day	1.85 ±0.47*	1.74 ±0.86*	3.95 ±1.30
T_{min} = minimum generation time (days)	43.2 ±6.76*	35.8 ±5.33*	28.0 ±2.9
T_c = cohort generation time (days)	52.8	60.6	47.4
$\sum m_x$ = summation of age-specific fecundity	24.4	59.8	78.2
$R_o = \sum U_x$ = net reproductive rate per generation	5.22	21.2	49.1
r_m = intrinsic rate of natural increase (d^{-1})	0.032	0.057	0.099

Table 4.4. Summary of results (mean ±1 SD) from cohort life-table experiments with *Tisbe battagliai* reared at 20°C and algal concentrations of 520, 1300 and 3250 µgC l^{-1} [* denotes a significant ($P < 0.05$) departure from a sex ratio of 1:1].

Parameter	Algal concentration (µgC l^{-1})		
	520	1300	3250
% Copepods that reached the adult stage	10 (n=5)	52 (n=26)	94 (n=47)
% Males	40 (n=2)	77 (n=20)*	68 (n=32)
% Females	60 (n=3)	23 (n=6)	32 (n=15)
% Egg-producing females	67 (n=2)	50 (n=3)	80 (n=12)
% Infertile females	33 (n=1)	50 (n=3)	20 (n=3)
Lifespan (days) of			
Males	45.0 ±8.48	32.5 ±7.25	41.3 ±9.96
Females	63.0 ±3.56	44.5 ±21.2	50.7 ±23.0
Egg-producing females	64.0 ±4.24	60.0 ±20	54.3 ±24.2
Infertile females	61.0 ±0	29.0 ±0	32.7 ±8.96
Reproductive period (days) of egg-producing females	34.0 ±12.7	37.3 ±21.1	21.6 ±16.5
No. of broods/ egg-producing females	7.00 ±4.24	8.00 ±6.56	6.42 ±4.32
No. of nauplii/ egg-producing females	85.5 ±72.8	126 ±125	157 ±141
Offspring per female per reproductive day	2.28 ±1.29	2.68 ±1.87	7.44 ±3.30
T_{min} = minimum generation time (days)	30.0 ±8.49	22.3 ±2.08	16.9 ±2.68
T_c = cohort generation time (days)	43.5	41.1	28.9
$\sum m_x$ = summation of age-specific fecundity	28.5	82.2	87.8
$R_o = \sum U_x$ = net reproductive rate per generation	7.12	15.8	59.9
r_m = intrinsic rate of natural increase (d^{-1})	0.047	0.077	0.182

Table 4.5. Summary of results (mean ±1 SD) from cohort life-table experiments with *Tisbe battagliai* reared at 25°C and algal concentrations of 520, 1300 and 3250 µgC l^{-1} [* denotes a significant ($P < 0.05$) departure from a sex ratio of 1:1].

Parameter	Algal concentration (µgC l^{-1})		
	520	1300	3250
% Copepods that reached the adult stage	0	44 (n=22)	80 (n=40)
% Males	0	82 (n=18)*	70 (n=28)
% Females	0	18 (n=4)	30 (n=12)
% Egg-producing females	-	50 (n=2)	92 (n=11)
% Infertile females	-	50 (n=2)	08 (n=1)
Lifespan (days) of			
Males	0	17.8 ±3.73	22.8 ±7.21
Females	0	17.8 ±1.26	24.7 ±6.87
Egg-producing females	-	17.5 ±2.12	25.3 ±6.86
Infertile females	-	18.0 ±0	18.0 ±0
Reproductive period (days) of egg-producing females	0	0	6.82 ±7.25
No. of broods/ egg-producing females	0	0	2.82 ±2.36
No. of nauplii/ egg-producing females	0	0	44.7 ±47.2
Offspring per female per reproductive day	0	0	9.29 ±4.58
T_{min} = minimum generation time (days)	0	0	12.1 ±1.04
T_c = cohort generation time (days)	0	0	19.2
$\sum m_x$ = summation of age-specific fecundity	0	0	46.1
$R_o = \sum U_x$ = net reproductive rate per generation	0	0	16.4
r_m = intrinsic rate of natural increase (d^{-1})	0	0	0.177

Table 4.6. Summary of results from cohort life-table experiments with *Tisbe battagliai* reared at 15, 20 and 25°C and algal concentrations of 0, 83, 208, 520, 1300 and 3250 µgC l^{-1} (n.c. values could not be calculated). In those treatments identified as *extinct*, all copepods had died or did not produce any offspring, therefore, populations were not only decreasing ($r_m < 0$) but became extinct.

Temp. (°C)	Algal conc. (µgC l^{-1})	Parameter			
		T_c	$\sum m_x$	R_o	r_m
15	83	n.c.	n.c.	0.063	-0.058
	208	n.c.	n.c.	0.069	-0.075
	520	52.8	24.4	5.22	0.032
	1300	60.6	59.8	21.2	0.057
	3250	47.4	78.2	49.1	0.099
20	83	0	0	0	*extinct*
	208	0	0	0	*extinct*
	520	43.5	28.5	7.12	0.047
	1300	41.1	82.2	15.8	0.077
	3250	28.9	87.8	59.9	0.182
25	83	0	0	0	*extinct*
	208	0	0	0	*extinct*
	520	0	0	0	*extinct*
	1300	0	0	0	*extinct*
	3250	19.2	46.1	16.4	0.177

Table 4.7. Demographic parameters of *Tisbe* spp (* lifespan of egg producing females only).

Species	Temp. (°C)	Salinity (‰)	Survival of nauplii to adulthood	Sex ratio (% female)	Mean lifespan			T_{min}	T_{coh}	R_o	r_m	References
					All	Male	Female					
Tisbe furcata wild	18	32	0.98	-	54.3	-	-	14.9	-	93.9	0.236	Bergmans (1984)
wild	18	32	0.98	-	52.9	-	-	16.9	-	91.1	0.197	
wild	18	32	0.87	-	48.3	-	-	14.5	-	172	0.279	
culture	18	32	0.97	-	58.7	-	-	17.2	-	184	0.241	
Tisbe furcata	18	32	0.98	47	-	-	-	14.9	-	93.9	0.233	Bergmans (1981)
Tisbe furcata	15	34	-	77	-	-	64.7*	20.7	-	67.5	0.166	Bechmann (1994)
Tisbe holothuriae	18	20	0.95	27	-	30.6	28.2	14.5	18.7	21.9	0.172	Bergmans &
	18	32	0.92	48	-	34.2	33.0	13.7	17.0	75.3	0.263	Janssens (1988)
Tisbe battagliai	18	20	0.88	51	-	30.0	30.7	16.2	21.0	58.6	0.203	
	18	32	0.73	68	-	31.1	31.4	18.2	21.9	61.6	0.196	
Tisbe battagliai	15	35	0.84	60	58.4	59.6	70.3	28.0	47.4	49.1	0.099	Present study
	20	35	0.94	32	42.7	41.3	50.7	16.9	28.9	59.9	0.182	
	25	35	0.80	30	20.3	22.8	24.7	12.1	19.2	16.4	0.177	

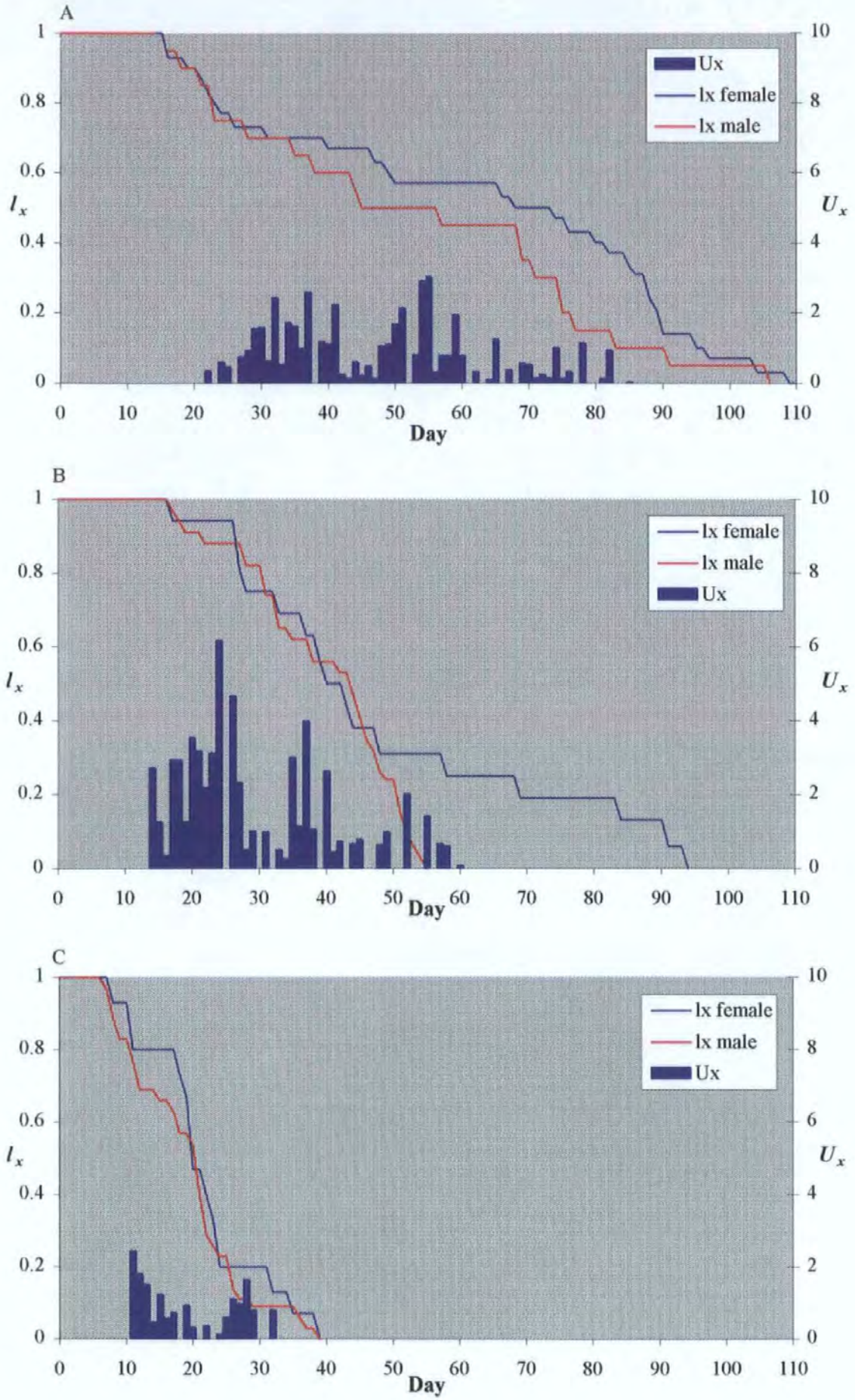


Figure 4.1. Age-specific survival (l_x) and fecundity (U_x) of *Tisbe battagliai* reared at temperatures of (A) 15°C, (B) 20°C and (C) 25°C.

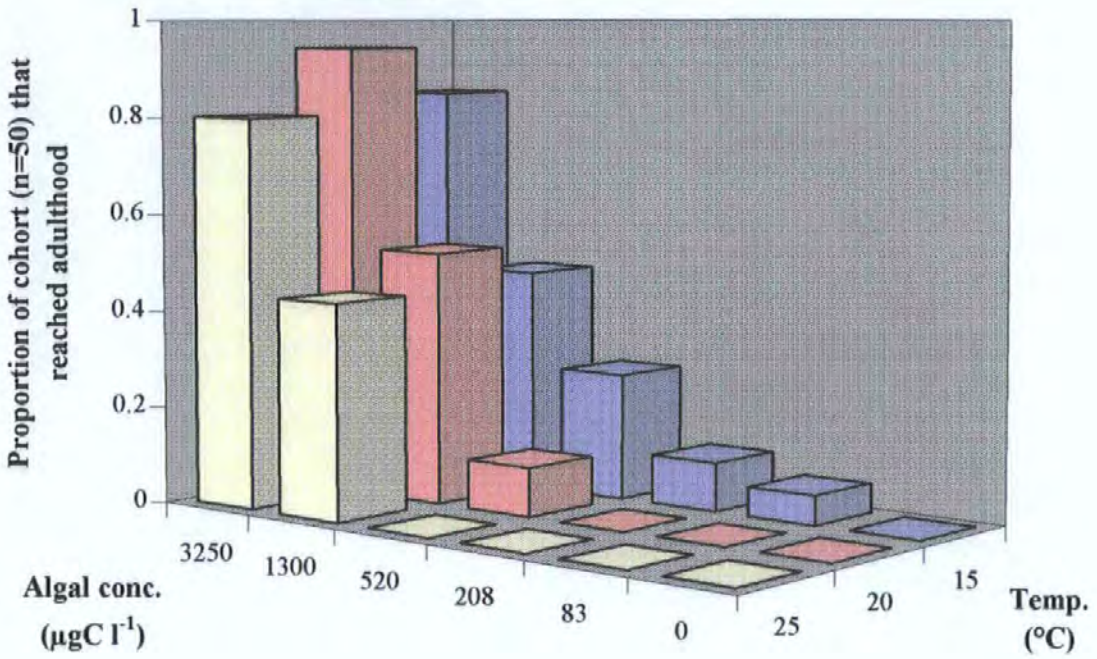


Figure 4.2. The effect of temperature (15, 20 and 25°C) and food concentration (0 - 3250 µgC l) on the development of *Tisbe battagliai* from hatching to the adult stage.

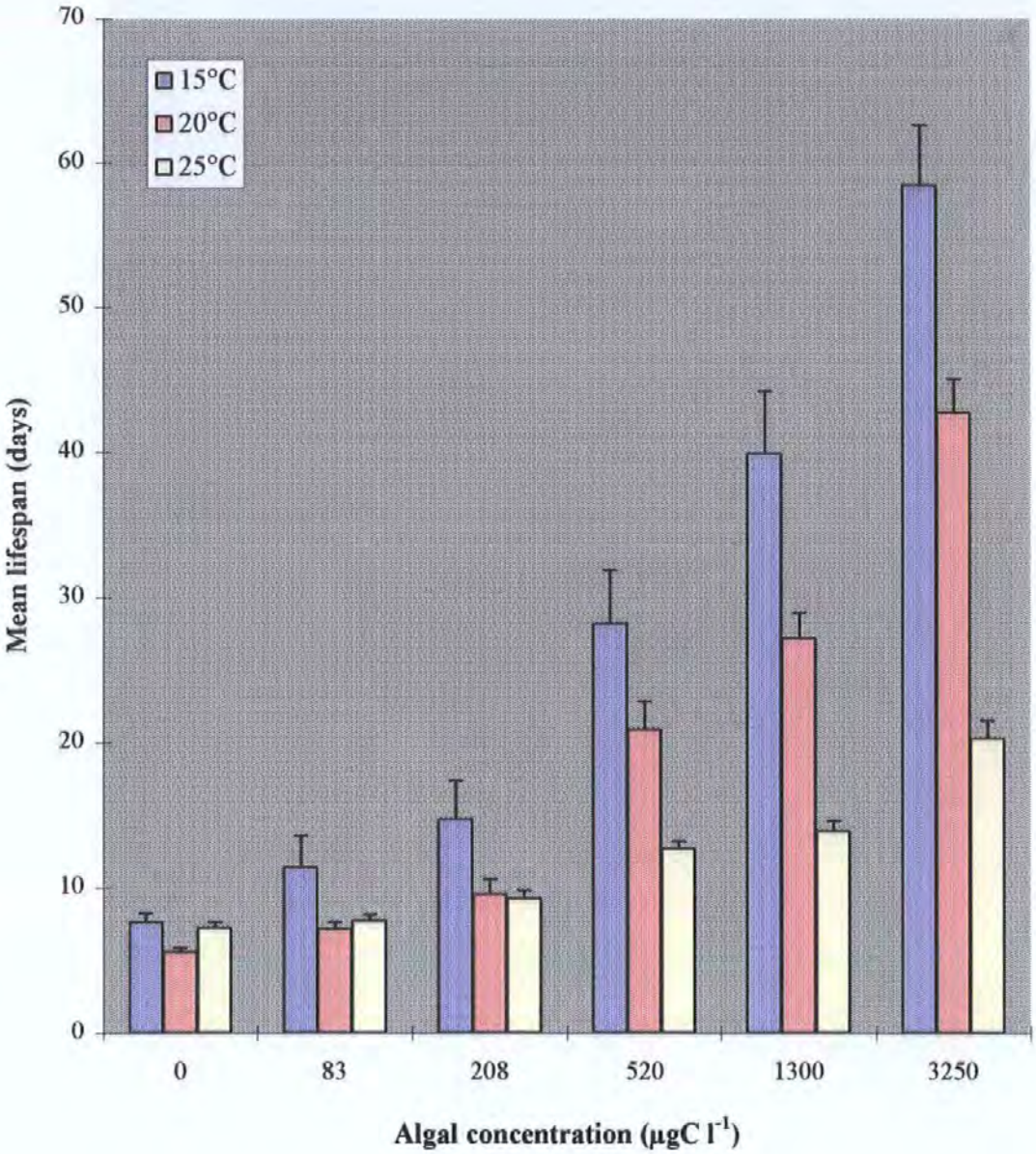


Figure 4.3. The effect of temperature (15, 20 and 25°C) and food concentration (0, 83, 208, 520, 1300 and 3250 µgC l) on cohort lifespan of *Tisbe battagliai*. Values represent the mean ±1SE.

CHAPTER 5

EFFECT OF FOOD QUALITY ON THE POPULATION DYNAMICS OF *TISBE BATTAGLIAI*

5.1. INTRODUCTION

Tisbe spp have been cultured successfully in the laboratory on a wide range of diets (Table 5.1). Synthetic foods have been used in monospecific diets (Gaudy & Guerin, 1977) but appear to be more effective as components of mixed diets containing natural food sources such as microalgae (Heath, 1994). Synthetic diets may be cheaper to prepare than culturing microalgae but they are not representative of food resources available in nature and the benefits associated with the use of these foods in laboratory culture may derive from specific components (e.g. vitamins) or as a substrate for microbial growth. Previous studies of the nutritional requirements of copepods have shown that specific dietary components (e.g. fatty acids) are important for reproduction (Provasoli, 1970). Copepods, and other marine invertebrates, have only a limited ability to synthesise long chain polyunsaturated fatty acids, therefore, these essential fatty acids must be supplied by their diet. Marine microalgae can synthesise long chain fatty acids, thus various algal species are considered important components in the diet of many species of copepods cultured in the laboratory. Consequently, microalgae remain the principal component of copepod diets and their success is determined largely by their biochemical composition, which has been shown to vary widely among different species (Brown *et al.*, 1989). In the laboratory, the choice of which algal species to use in copepod diets is governed by the need to ensure adequate quantity and nutritional quality, and the extent to which the ingested food meets the nutritional requirements of the organism. Algal diets are time consuming to prepare and the requirement for mixed species or unialgal diets will influence the time taken to culture the algae. Although nutritional quality is the prime concern in copepod diets, the development of cost-effective culture and toxicity test procedures is an important consideration in regulatory ecotoxicology.

Tisbe battagliai has been cultured for extended periods in the laboratory on a unialgal diet of *Isochrysis galbana* and this alga appears to meet the nutritional requirements for postembryonic development and reproduction in this species (Chapters 2 and 3). Previous chapters have established the relative importance of food quantity on the population dynamics of *T. battagliai* (Chapters 2 - 4), but questions concerning food quality, and the ability of *I. galbana* to provide an optimal source of nutrition, remain unresolved. A review of the literature suggests that the long-term health and fecundity of copepods benefit from mixed food diets (Hicks & Coull, 1983; Heath, 1994). Mixed diets seem best able to fulfil the nutritional requirements for high levels of offspring production, probably due to the better provision of trace elements and vitamins. Diet has also been shown to influence the results of toxicity tests and test precision (Cowgill, 1986; Lee *et al.*, 1986). Maintaining consistent and adequate performance in terms of survival, development and reproduction of test organisms presents a significant challenge to the development of successful chronic toxicity test procedures.

The objective of the following series of experiments was to establish the effect of food quality on the population dynamics of *Tisbe battagliai*. Mixed species and unialgal diets consisting of *Isochrysis galbana* and *Rhodomonas reticulata* were chosen to represent different sources of nutrition for the copepods. The effect of these algal diets on postembryonic development, reproduction and the complete life cycle of *T. battagliai* was investigated, and the results compared with those from previous experiments using a unialgal diet of *I. galbana* (Chapters 2 - 4).

5.2. MATERIALS AND METHODS

The test methods follow the procedures used to investigate the effect of food quantity on postembryonic development, reproduction and the complete life cycle (life-table analysis) of *Tisbe battagliai* (Chapters 2, 3 and 4 respectively). Only a brief summary of the methods is reported here, highlighting differences from the previous protocols. Food quality experiments were conducted at optimal temperature (20°C) and food concentration (3250 µgC l^{-1}) for development and reproduction of *T. battagliai* (Chapters 2 and 3 respectively).

5.2.1. Algal culture

Rhodomonas reticulata (Strain CCAP 995/2, Culture Centre of Algae and Protozoa) was grown in a chemostat using the same procedures used to culture *Isochrysis galbana* (Chapter 2). Hereafter, the two algae will be referred to by their generic names only. Cell density was maintained at ≈ 5 x 10^6 cells ml^{-1} by a 30% daily replacement of the culture volume. Culture outflow was pumped from the reaction vessel and the cells collected daily for preparation of food suspensions. *Rhodomonas* cells settled to the base of the collection vessel and, after the supernatant was carefully decanted (by siphon tube), were resuspended in filtered (0.2 µm) seawater to provide a stock suspension containing 3 x 10^7 cells ml^{-1}.

5.2.2. Test procedure

Stock suspensions of algae were diluted with filtered (0.2 µm) seawater to provide concentrations of 3250 µgC l^{-1}, consisting of *Isochrysis* and *Rhodomonas* in the following proportions (%); 100:0, 75:25, 50:50, 25:75 and 0:100. Carbon values and cell concentrations for the freshly prepared algal stock suspensions were determined using a Dohrman DC-190 high temperature carbon analyser and a Model ZB Coulter Counter respectively. Algal stock

suspensions were stored in a refrigerator in the dark and used within 7 days. Separate experiments were carried out to determine the effect of algal diet on postembryonic development, reproduction and the complete life cycle (life-table analysis) of *Tisbe battagliai*. Experiments to determine the effect of algal diet on postembryonic development were started with < 2 h old nauplii, reproduction experiments with 12 d old first egg sac females, and life-table analyses with < 7 h old nauplii.

5.2.3. Statistical analysis

Statistically significant effects of algal diet on development time, female lifespan, reproductive period, number of broods, number of offspring and number of offspring per female per reproductive day were calculated using analysis of variance techniques (Sokal & Rohlf, 1995). Data that failed to meet the assumptions for analysis of variance (normality and equality of variances) were analysed with non-parametric (distribution-free) techniques (Hollander & Wolfe, 1973). In life-table experiments, the proportion of nauplii that reached the adult stage in each cohort was examined using a contingency table procedure (Hayslett & Murphy, 1976) to identify significant differences between the diets. Statistical comparisons of the data from different algal diets were made against the standard unialgal diet of *Isochrysis* used in previous experiments (Chapters 2 - 4).

5.3. RESULTS

5.3.1. Postembryonic development

The effect of algal diet on postembryonic development of *Tisbe battagliai* is shown in Table 5.2. No attempt was made to statistically analyse the data for the separate sexes due to the small number of copepods involved. Instead, statistical comparisons between the different

diets were made using the development times for the combined naupliar stages (N1-N6), combined copepodid stages (C1-C5) and the time from hatching to adult for both sexes combined. There was a significant reduction in development time from hatching to adult when the unialgal diet of *Isochrysis* was supplemented with, or replaced by, *Rhodomonas* (Table 5.2). The same results were observed for the combined copepodid stages but differences in development times for the combined naupliar stages were not significant.

5.3.2. Reproduction

The unialgal and mixed algal diets had different effects on the reproduction of *Tisbe battagliai* (Table 5.3). When *Isochrysis* was replaced by, or supplemented with, *Rhodomonas* there were significant increases in lifespan, total number of broods, total number of offspring and the number of offspring per female per reproductive day (Fig. 5.1). The reproductive period (expressed as the total number of female reproductive days) was not affected by diet (Fig. 5.1B; Table 5.3). Results were related to the proportion of *Rhodomonas* in the diet, such that diets containing high proportions (75% and 100%) of *Rhodomonas* resulted in significant increases in lifespan and the total number of broods. Diets containing 25% or more of *Rhodomonas* resulted in significant increases in the total numbers of offspring and the number of offspring per female per reproductive day (Table 5.3). Offspring production tended to increase with successive increases in the proportion of *Rhodomonas* in the diet and significant differences corresponded to a 1.8 - 2.7 times increase in offspring numbers (Fig. 5.2). There was a general trend of larger brood size and a reduction in the interval of time between the hatching of successive broods (interbrood duration) with increasing proportion of *Rhodomonas* in the algal diet (Fig. 5.3). Statistical comparison of differences in brood size and interbrood duration revealed significant differences (Tables 5.4 and 5.5). First brood values were excluded from the analyses as these were not produced under the influence of the test

diets. Within each diet, relatively few females produced more than 6 broods, therefore, only data for broods 2 - 5 were chosen for analysis to provide the maximum number of data points for statistical comparison. Compared with *Isochrysis* as the sole dietary alga, brood size was significantly higher, and interbrood duration significantly shorter, on diets containing a mixture of *Isochrysis* and *Rhodomonas*, and *Rhodomonas* alone (Tables 5.4 and 5.5 respectively). Within each diet, brood size appeared to stay relatively constant for a period before gradually declining.

5.3.3. Complete life cycle (life-table analysis)

The demographic data for *Tisbe battagliai* reared on the different algal diets are shown in Table 5.6. These data were calculated from daily observations of survivorship and fecundity of *T. battagliai* nauplii from hatching through to day at death. When the *Isochrysis* diet was replaced by, or supplemented with relatively high proportions (50% & 75%) of *Rhodomonas*, there were significant increases in the number of copepods reaching the adult stage, significant reductions in the minimum generation time, and significant increases in the number of nauplii per female and offspring per female per reproductive day (Table 5.6). With the smallest proportion of *Rhodomonas* (25%) in the diet, only the minimum generation time showed a significant change (decrease) compared with the *Isochrysis* diet. The reductions in demographic parameters contributed to a reduction in the intrinsic rate of natural increase (r_m). Compared with the *Isochrysis* diet, there was a successive increase in r_m with increasing proportions of *Rhodomonas* in the algal diet and these increases were attributed to a reduction in the age at first reproduction and increased fecundity (Table 5.6). These increases were most pronounced for diets containing high proportions (75% & 100%) of *Rhodomonas* and amounted to a 2.2 - 2.4 times increase in r_m. The reproductive output (number of nauplii per female and offspring per female per reproductive day) of copepods fed the *Isochrysis* diet was

the lowest of all the diets examined, however, the net reproductive rate (R_o) indicated a 19 fold increase in population size from one generation to another. On the other hand, the net reproductive rate (R_o) for those diets providing maximum reproductive output (75% & 100% *Rhodomonas*) showed a 101 - 103 fold increase in population size from one generation to another. Trends in survivorship and reproduction of copepods fed the different algal diets are shown in Figure 5.4. The survivorship curves show a gradual increase in male and female mortality with time. Daily measurements of fecundity (U_x, the number of new-born females produced per day, per new-born female of the preceding generation) show reductions in the time of first reproduction, and increases in offspring production, with increasing proportions of *Rhodomonas* in the diet.

5.4. DISCUSSION

The rate of development and fecundity of *Tisbe battagliai* improved significantly when an algal diet consisting of *Isochrysis galbana* was supplemented with, or replaced by, *Rhodomonas reticulata*. Results suggest that the nutritional value of these algae for *T. battagliai* may be different and the potential reasons for differences in development and fecundity are explored below.

Improvements in development and fecundity may be due to potential differences in the biochemical composition and nutritional value of these algae for *T. battagliai*. Generally, the nutritional value of algal species has been assessed from fatty acid distribution (% of total fatty acid) and by the content of polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3). Evidence has accumulated that the quality, as well as the quantity, of algal lipids is very important in the nutrition of marine organisms. Many species of microalgae contain high proportions of 20:5n-3 and 22:6n-3

(Volkmann *et al.*, 1989), and these polyunsaturated fatty acids have been shown to have an essential role in the nutrition of bivalve molluscs (Langdon & Waldock, 1981; Webb & Chu, 1982), penaeid shrimps (Kanazawa *et al.*, 1979) and larval fish (Witt *et al.*, 1984; Stottrup & Attramadal, 1992). Copepods, and other marine invertebrates, have the ability to biosynthesise 20:5n-3 and 22:6n-3 fatty acids from shorter-chain PUFAs such as linolenic acid, 18:3n-3 (Sargent & Henderson, 1986) although rates are reported to be low and these fatty acids have, therefore, been considered essential dietary components for marine copepods (Fraser *et al.*, 1989).

The nutritional value of different algal species is probably due to their chemical composition which varies widely amongst different species (Mourente *et al.*, 1990). In laboratory cultured algae, the chemical composition (e.g. lipid classes) can change with nutrient availability, age of culture and growth conditions (Shamsudin, 1992; Molina Grima *et al.*, 1993) and these changes may, in turn, affect their nutritional value (Thompson *et al.*, 1993). For example, the addition of a vitamin mixture to the algal culture medium restored normal fertility, at least for four generations, in *Tigriopus japonicus* when the food organism was either *Isochrysis galbana* or *Chroomonas* spp (Shiraishi & Provasoli, 1959). In relation to fatty acid composition, experimental evidence suggests that 20:5n-3 and 22:6n-3, and the combination of these polyunsaturated fatty acids in the diet, play an important role in copepod reproduction. *Isochrysis galbana* is relatively rich in 22:6n-3 but deficient in 20:5n-3, which is reported to be the major polyunsaturated fatty acid in *Rhodomonas* spp (Stottrup & Jensen, 1990). The nutritional benefits derived from the addition of *Rhodomonas* to the diet may be related to the fatty acid composition, perhaps the provision of 20:5n-3, but other factors (e.g. vitamins and trace elements) may be equally important in determining the nutritional value of the algal food source.

Microalgae are used widely in mariculture as a preferred natural food for many marine organisms including bivalve molluscs and brine shrimp, and as a primary food source for fish

larvae (De Pauw & Persoone, 1988). Not all algal species, however, are equally successful in supporting good growth and survival for all cultured species (Epifanio *et al.*, 1981; Webb & Chu, 1983; Enright *et al.*, 1986), and differences in the size, digestibility and biochemical composition of the microalgae may account for this (Webb & Chu, 1983). In relation to the biochemical composition, the algal nutritional quality may depend not only on the major biochemical fractions (protein, carbohydrate and lipid) but also on the levels of specific amino acids, fatty acids, sugars, sterols, vitamins and minerals (Brown *et al.*, 1989). Major differences in the sugar composition of the polysaccharides of algal species have been reported (Brown, 1991), and laboratory culture and harvest regimes can produce significant differences in the proportions of protein and carbohydrate (Brown *et al.*, 1993). The environmental conditions under which algae are cultured dictate their biochemical composition and may affect their nutritional value. Consequently, manipulation of culture conditions, and harvesting at specific growth phases, may enable the biochemical composition of the microalgal cultures to be tailored for specific purposes (Dunstan *et al.*, 1993). Bacteria have been shown to be important dietary components for some species of *Tisbe* (Brown & Sibert 1977; Rieper 1978; Vanden Berghe & Bergmans, 1981) and there is supporting evidence that the success of some algal diets may be attributed to the supplemental importance of microbial sources, either in the algae added as food or in the dilution water. For example, Provasoli *et al.* (1959), using bacteria-free cultures, found that multiple generations of *Tigriopus japonicus* were achieved only in the presence of a bacterial flora.

Laboratory studies have shown that the biochemical composition of algae can vary widely, depending on environmental conditions (Sukenik & Wahnon, 1991; Dunstan *et al.*, 1993; Thompson *et al.*, 1993; Molina Grima *et al.*, 1994; Reitan *et al.*, 1994; Renaud *et al.*, 1995). During the present study, attempts were made to ensure that the algal culture conditions were constant, however, temporal variability in the algal culture conditions, and

subsequent effects on the biochemical composition and nutritional value of the algal diet for *Tisbe*, could not be ruled out. Such variations may explain why the nutritional value of the high ration (3250 μgC l^{-1}) *Isochrysis* diet used here was relatively poor (measured by offspring production) compared with previous experiments (Chapter 3).

Evidence to support the dietary importance of fatty acid composition for copepod reproduction is provided by several authors. The number of eggs produced by *Calanus finmarchicus* is influenced by previous dietary history and, specifically, by the total lipid content of the phytoplankton (Gatten *et al.*, 1980). More recent studies have emphasised that the distribution of lipids, not the total lipid content, is more important in determining the nutritional value of specific algal diets. The rate of egg production of *Acartia tonsa* fed five different unialgal diets (two diatoms and three flagellates) was highest when fed *Isochrysis galbana* (T-ISO) and *Rhodomonas baltica* (Stottrup & Jensen, 1990). Although there was no clear relationship between egg production and the quantity of dietary PUFAs, there was a strong negative correlation between the ratio of 20:5n-3 and 22:6n-3. Stottrup & Jensen (1990) reported that egg production rates were highest for copepods fed *Isochrysis* and *Rhodomonas* (ratios of 0.034 and 1.6 respectively) and lower for the diatoms *Thalassiosira weissflogii* and *Ditylum brightwellii* (ratios of 4.5 and 8.0 respectively). *Dunaliella tertiolecta*, which is deficient in 20:5n-3 and 22:6n-3, was a very poor diet for *A. tonsa* and copepods refused to feed on this species after some time (Stottrup & Jensen, 1990). Jonasdottir (1994) found that the protein content of the algal diet affected the rate of egg production in *A. tonsa* and *A. hudsonica* but the chemical constituents providing the best correlation with egg production rates were the lipid fraction of the phytoplankton. Egg production rates were highest for copepods fed *Rhodomonas lens* and a highly significant negative correlation was recorded for the ratio of 20:5n-3 and 22:6n-3 (ratio of 1.5 - 2.0 for *R. lens*). More recently, Jonasdottir and Kiorboe (1996) reported correlations between fatty acid

composition in the diet and egg production and hatching in *A. tonsa*, indicating that these reproductive events are affected by the nutritional quality of the food. The data by Jonasdottir and Kiorboe (1996) are in agreement with Stottrup & Jensen (1990) and suggest that highly unsaturated fatty acids, in the right combination, are important for egg production in copepods. The fatty acid distribution in adult *Tisbe holothuriae* reared on unialgal diets of *Dunaliella tertiolecta* and *Rhodomonas baltica* reflected largely their diet, but the presence of 20:5n-3 and 22:6n-3 PUFAs in newly-hatched nauplii were similar regardless of the presence of these fatty acids in the diet (Norsker and Stottrup, 1994). It appears that *Tisbe* can elongate dietary n-3 PUFAs (possibly from 18:3n-3) at significant rates although the process might still be rate limiting for reproduction, in which case the dietary supply of these fatty acids might enhance nauplii production.

Offspring production by *Tisbe battagliai* increased with increasing proportions of *Rhodomonas* in the diet, however, the results for mixed diets (*Isochrysis* and *Rhodomonas*) were not significantly better than those for *Rhodomonas* alone. Elsewhere in this discussion, it is proposed that the biochemical composition of *Rhodomonas* may provide clues to these improvements in fecundity of *T. battagliai* but it is not known what constituents (e.g. fatty acids, vitamins) are responsible. Compared with results from earlier experiments (Chapter 3), the nutritional value of *Isochrysis* (measured by offspring production) in the current series of experiments was inferior. This reduction in offspring production indicates the potential limitation of unialgal diets in that the key constituents in the algal diet important for reproduction may not always be present in sufficient quantity or quality. The finding that *Rhodomonas* is better than *Isochrysis* as a unialgal diet should be treated with caution. Nutritional deficiencies in the *Rhodomonas* diet may only become evident after a longer period of time, therefore, multigeneration culture of copepods would be required to establish the suitability of *Rhodomonas* for long-term culture of *Tisbe*. Furthermore, the copepods used in

these experiments were derived from cultures fed with a unialgal diet of *Isochrysis*, therefore, the results may have been influenced by their previous feeding regime. In view of the potential limitations posed by unialgal diets, laboratory culture and toxicity test procedures for *T. battagliai* may benefit from a mixed algal diet of *Isochrysis* and *Rhodomonas*. Results show that increases in development rate and fecundity occurred at proportions of 50:50% and 25:75% *Isochrysis*:*Rhodomonas*. It would be advisable to proceed initially with a mixed algal diet of 50:50% *Isochrysis*:*Rhodomonas* for the culture of *T. battagliai* until the potential long-term effects of diets containing high levels of *Rhodomonas* are established.

Algal diet had a significant effect on the fertility and reproduction of *Tisbe battagliai*. These findings are in general agreement with published studies that highlight the importance of algae for egg production by calanoid and harpacticoid copepods. For calanoid copepods, egg production in *Acartia tonsa* was influenced by the quantity and quality of the algal food source (Stottrup & Jensen, 1990), and the number of eggs produced by *Calanus helgolandicus* was influenced by the total lipid content of the food (Gatten *et al.*, 1980). Kiorboe *et al.* (1985) suggested that lipids were important for egg production in *A. tonsa*, while protein was important for somatic growth of nauplii and copepodid stages. The reproductive success of *A. tonsa* and *A. hudsonica* was correlated with protein content and especially the specific fatty acid component of the algal diet (Jonasdottir, 1994). Detrital foods supplied the majority of the energy needs of the harpacticoid *Scottolana canadensis*, however, the addition of algal cells was necessary for egg production (Heinle *et al.*, 1977). Several authors have noted the benefits of mixed algal diets for offspring production in harpacticoids. Egg production by *Euterpina acutifrons* was between 2 - 20 times higher on a mixed algal diet (5 species) than either algal species fed separately (Nassogne, 1970). Zurlini *et al.* (1978) observed that the highest numbers of eggs produced by *E. acutifrons* occurred for copepods fed the greater number of algal species (four, instead of three algal species). A mixture of *Isochrysis galbana* and *Thalassiosira pseudonana* resulted in higher offspring

production by *Scottolana canadensis* than when either algae was fed individually (Harris, 1977). Provasoli *et al.* (1959) and Shiraishi & Provasoli (1959) found that *I. galbana* was able to support only eight generations of the harpacticoid *Tigriopus japonicus*, although populations were still thriving after 28 generations on a mixture of *I. galbana* and *Rhodomonas lens*. These findings confirm the use of mixed algal diets for the laboratory culture of copepods. For *Tisbe battagliai*, unialgal diets of *Isochrysis galbana* and *Rhodomonas reticulata* meet the nutritional requirements for development and reproduction, however, a mixture of these two species may minimise the risk that key nutritional components will be limiting or absent.

Table 5.1. Food sources used in the laboratory culture of *Tisbe battagliai* and *Tisbe holothuriae* († indicates *T. battagliai* only; †† indicates *T. battagliai* and *T. holothuriae*; all other studies are for *T. holothuriae* only).

Food	Salinity (‰)	Temp. (°C)	Reference
Fish muscle, bacteria, wheat grains, rabbit erythrocytes			Lwoff (1927)
Ulva sp., wheat germ, *Phaeodactylum* sp., *Nitzschia* sp.	38	18	Battagliai (1970)
Dried *Mytilus edulis*	30	22	Hoppenheit (1975)
Benthic diatoms growing on a red alga, *Pachymeniopsis elliptica*	34	25	Park (1976)
Tetramin, Bioter food, Renutryl	38	19	Gaudy & Guerin (1977)
Dried *Mytilus edulis*, bacteria	28 - 30	18	Reiper (1978)
Ulva sp., boiled wheat	34 - 36	18	Fava & Crotti (1979)
Dunaliella sp., bacteria		18	Vanden Berghe & Bergmans (1981)††
Renutryl, Germalyne	28 - 38	10 - 24	Gaudy & Guerin (1982)
Wheat pericarps	35	18	Brand (1985)
Wheat pericarps, *Dunaliella* sp.	35	18	Brand *et al.* (1986)
Dried lettuce, *Dunaliella* sp.	20, 32	18	Bergmans & Janssens (1988)††
Boiled wheat, *Dunaliella* sp.	20, 25, 35	18	Fava & Martini (1988)
Boiled wheat, *Dunaliella* sp.	20, 25, 35	18	Fava & Fusari (1991)
Tetramin, 3 bacterial strains	38	19 - 20	Guerin & Rieper-Kircher (1991)
Ulva sp., Fryfood	26, 32, 38, 44	14, 19, 24	Miliou & Moraitou-Apostolopoulou (1991a)
Ulva sp., Fryfood, dried *Mytilus edulis*, Soya, Yeast, *Spirullina* sp.	38	19	Miliou & Moraitou-Apostolopoulou (1991b)
Dried *Mytilus edulis*, *Dunaliella* sp., *Skeletonema* sp.	28	20	Zhang & Uhlig (1991)
Isochrysis sp.	35	15, 20, 25	Williams & Jones (1994)†
Dunaliella sp., frozen shrimp solids	28 - 33	18	Norsker & Stottrup (1994)
Nannochloris sp. dried *Mytilus edulis*, salmon pellet	30	20	Heath (1994)

Table 5.2. Mean stage durations in days (±1SD) of the combined naupliar stages (N1-N6), combined copepodid stages (C1-C5), and hatching to adult (N1-A) for *Tisbe battagliai* reared at 20°C and fed 3250 μgC l^{-1} of an algal diet consisting of different proportions (%) of *Isochrysis galbana* and *Rhodomonas reticulata* [* denotes a significant difference ($P < 0.05$) compared with the 100:0 diet].

% *Isochrysis: Rhodomonas*	n	Naupliar stages (N1 - N6)	Copepodid stages (C1 - C5)	Hatching to adult (N1 - A)		
100:0	10	4.00 ±0.47	5.80 ±0.92	9.40 ±0.53	(n=7)	male
				10.7 ±0.58	(n=3)	female
				9.80 ±0.79		both
75:25	10	4.00 ±0	5.00 ±0*	9.00 ±0	(n=8)	male
				9.00 ±0	(n=2)	female
				9.00 ±0*	-	both
50:50	9	4.00 ±0	5.10 ±0.30*	9.00 ±0	(n=4)	male
				9.20 ±0.40	(n=5)	female
				9.10 ±0.30*	-	both
25:75	8	3.88 ±0.35	5.40 ±0.52*	8.75 ±0.50	(n=4)	male
				9.75 ±0.50	(n=4)	female
				9.25 ±0.71*	-	both
0:100	10	4.00 ±0	5.00 ±0*	9.00 ±0	(n=5)	male
				9.00 ±0	(n=5)	female
				9.00 ±0*	-	both

Table 5.3. The effect of algal diet on aspects of the reproduction of *Tisbe battagliai*. Copepods (n=10) were reared at 20°C and fed 3250 µgC l^{-1} of an algal diet consisting of different proportions of *Isochrysis galbana* and *Rhodomonas reticulata* [* denotes a significant difference ($P < 0.05$) compared with the 100:0 diet; [a] The number of female reproductive days was calculated as the total number of days between release of the first and last broods of offspring; [b] Excluding the first brood of offspring].

% *Isochrysis*: *Rhodomonas*	Lifespan (days)			Total no. of female reproductive days[a]			Total no. of broods[b]			Total no. of offspring[b]			Offspring per female per reproductive day[b]		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
100:0	30.9	4.5	26-40	21.6	5.2	10-28	5.1	1.3	3-7	104	28	57-143	5.1	1.9	2.0-8.5
75:25	30.9	4.9	26-39	17.9	4.3	10-21	5.4	1.3	3-7	163	57	72-248	9.1*	2.2	5.2-11.8
50:50	33.8	3.0	28-39	17.5	4.1	11-25	5.6	1.6	3-8	215*	85	57-363	12.4*	4.1	3.0-18.2
25:75	40.9*	8.7	27-58	22.0	10.4	11-38	6.9*	2.4	4-11	256*	81	123-343	13.0*	3.9	5.4-17.5
0:100	38.2*	7.4	29-48	21.9	5.8	19-27	7.5*	2.0	6-10	284*	106	143-375	12.7*	2.9	7.5-16.6

Table 5.4. The effect of algal diet on the brood size of *Tisbe battagliai*. Copepods (n=10) were reared at 20°C and fed 3250 µgC l^{-1} of an algal diet consisting of different proportions of *Isochrysis galbana* and *Rhodomonas reticulata*. Results are expressed as the mean ±1SD and * denotes a significant difference ($P < 0.05$) in brood size (analysis of broods 2-5 only; see Section 5.3.2) compared with the 100:0 diet.

% Isochrysis:	Brood number											
Rhodomonas	1	2	3	4	5	6	7	8	9	10	11	12
100:0	17 ±6.7 (n=10)	20 ±4.8 (n=10)	27 ±8.8 (n=10)	26 ±10 (n=10)	17 ±7.1 (n=9)	15 ±6.4 (n=7)	18 ±9.6 (n=3)	13 ±9.2 (n=2)	-	-	-	-
75:25	15 ±7.0 (n=10)	31 ±6.4* (n=10)	36 ±11 (n=10)	29 ±10 (n=10)	41 ±10* (n=9)	25 ±10 (n=8)	18 ±16 (n=5)	10 ±1.4 (n=2)	-	-	-	-
50:50	16 ±5.0 (n=10)	35 ±11* (n=10)	51 ±8.5* (n=10)	44 ±8.4* (n=9)	42 ±8.2* (n=9)	41 ±15 (n=7)	21 ±18 (n=6)	24 ±16 (n=4)	9 ±0 (n=1)	-	-	-
25:75	13 ±3.8 (n=10)	36 ±8.6* (n=10)	55 ±10* (n=10)	46 ±7.5* (n=10)	38 ±11* (n=10)	42 ±11 (n=7)	32 ±17 (n=7)	28 ±17 (n=6)	18 ±12 (n=5)	14 ±15 (n=2)	8 ±0 (n=1)	1 ±0 (n=1)
0:100	18 ±6.3 (n=10)	39 ±7.0* (n=10)	52 ±16* (n=10)	42 ±17* (n=9)	46 ±9.2* (n=9)	41 ±10 (n=9)	35 ±20 (n=9)	34 ±18 (n=7)	26 ±17 (n=5)	12 ±9 (n=4)	7 ±0 (n=1)	-

Table 5.5. The effect of algal diet on the interval of time (days) between hatching of successive broods of offspring by *Tisbe battagliai*. Copepods (n=10) were reared at 20°C and fed 3250 µgC l^{-1} of an algal diet consisting of different proportions of *Isochrysis galbana* and *Rhodomonas reticulata*. Results are expressed as the mean ±1SD and * denotes a significant difference ($P < 0.05$) in brood size (analysis of broods 2-5 only; see Section 5.3.2) compared with the 100:0 diet.

% Isochrysis:	Brood number										
Rhodomonas	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	10-11	11-12
100:0	5.6 ±2.4 (n=10)	3.5 ±0.7 (n=10)	3.7 ±0.8 (n=10)	4.1 ±0.6 (n=9)	4.3 ±1.8 (n=7)	4.0 ±1.0 (n=3)	4.5 ±0.7 (n=2)	-	-	-	-
75:25	3.6 ±0.7* (n=10)	3.1 ±0.6 (n=10)	3.2 ±0.6 (n=10)	3.0 ±0.5* (n=9)	3.5 ±1.2 (n=8)	3.2 ±0.5 (n=5)	4.5 ±2.1 (n=2)	-	-	-	-
50:50	3.1 ±0.6* (n=10)	2.5 ±0.7* (n=10)	3.1 ±0.6 (n=9)	2.8 ±0.7* (n=9)	2.6 ±0.5 (n=7)	2.8 ±0.8 (n=6)	3.3 ±0.5 (n=4)	4.0 ±0 (n=1)	-	-	-
25:75	2.9 ±0.7* (n=10)	2.4 ±0.5* (n=10)	3.1 ±0.3 (n=10)	2.4 ±0.5* (n=10)	3.0 ±0.6 (n=7)	3.1 ±0.7 (n=7)	3.4 ±0.9 (n=5)	3.2 ±0.8 (n=5)	4.5 ±2.1 (n=2)	3.0 ±0 (n=1)	4.0 ±0 (n=1)
0:100	3.0 ±0.5* (n=10)	2.5 ±0.5* (n=10)	2.6 ±0.5* (n=10)	2.7 ±0.5* (n=9)	2.9 ±0.3 (n=9)	2.9 ±0.6 (n=9)	3.1 ±0.4 (n=7)	2.8 ±0.5 (n=5)	3.0 ±0 (n=4)	3.0 ±0 (n=1)	-

Table 5.6. Summary of results (mean ±1 SD) from cohort life-table experiments with *Tisbe battagliai* reared at 20°C and 3250 µgC l^{-1}. Unless indicated otherwise, values represent mean ±1SD [* significant difference ($P < 0.05$) compared with the 100% *Isochrysis* diet; † significant difference compared with remaining diets].

Parameter	Algal diet (% *Isochrysis:Rhodomonas*)				
	100:0 (n=12)	75:25 (n=14)	50:50 (n=19)	25:75 (n=24)	0:100 (n=20)
% Copepods that reached the adult stage	60 (n=30)†	78 (n=39)†	96 (n=48)	100 (n=50)	100 (n=50)
% Males	50 (n=15)	61.5 (n=24)	54.2 (n=26)	50 (n=25)	60 (n=30)
% Females	50 (n=15)	38.5 (n=15)	45.8 (n=22)	50 (n=25)	40 (n=20)
% Egg-producing females	80 (n=12)	93.3 (n=14)	86.4 (n=19)	96 (n=24)	100 (n=20)
% Infertile females	20 (n=3)	06.7 (n=1)	13.6 (n=3)	04 (n=1)	0
Lifespan (days) of					
Males	49.3 ±18.6	56.3 ±14.4	57.0 ±14.3	52.5 ±17.2	57.4 ±2.99
Females	56.7 ±11.5	50.1 ±12.3	56.9 ±13.8	57.8 ±11.0	49.1 ±2.86
Egg-producing females	60.8 ±8.29	50.6 ±12.6	59.7 ±11.8	59.3 ±8.46	49.1 ±2.86
Infertile females	40.3 ±7.09	43.0 ±0	39.3 ±14.2	23.0 ±0	-
Reproductive period (days) of egg-producing females	25.5 ±9.02	23.5 ±13.4	31.2 ±13.3	20.9 ±11.9	19.5 ±18.39
No. of broods/ egg-producing females	6.25 ±2.09	5.43 ±3.18	9.00 ±3.67*	7.54 ±3.30	6.55 ±2.21
No. of nauplii/ egg-producing females	81.8 ±46.4	90.2 ±57.0	182 ±88*	210 ±108*	204 ±86*
Offspring per female per reproductive day	3.24 ±1.31	4.24 ±2.15	6.38 ±3.22*	10.8 ±3.69*	11.2 ±3.88*
T_{min} = minimum generation time (days)	27.6 ±4.44	21.4 ±1.95*	19.5 ±3.10*	18.2 ±2.68*	16.4 ±1.09*
T_c = cohort generation time (days)	36.2	29.9	33.2	27.7	25.1
$\sum m_x$ = summation of age-specific fecundity	37.9	51.5	91.1	105	110
$R_o = \sum U_x$ = net reproductive rate per generation	19.3	32.0	75.4	101	103
r_m = intrinsic rate of natural increase (d^{-1})	0.090	0.136	0.170	0.199	0.218

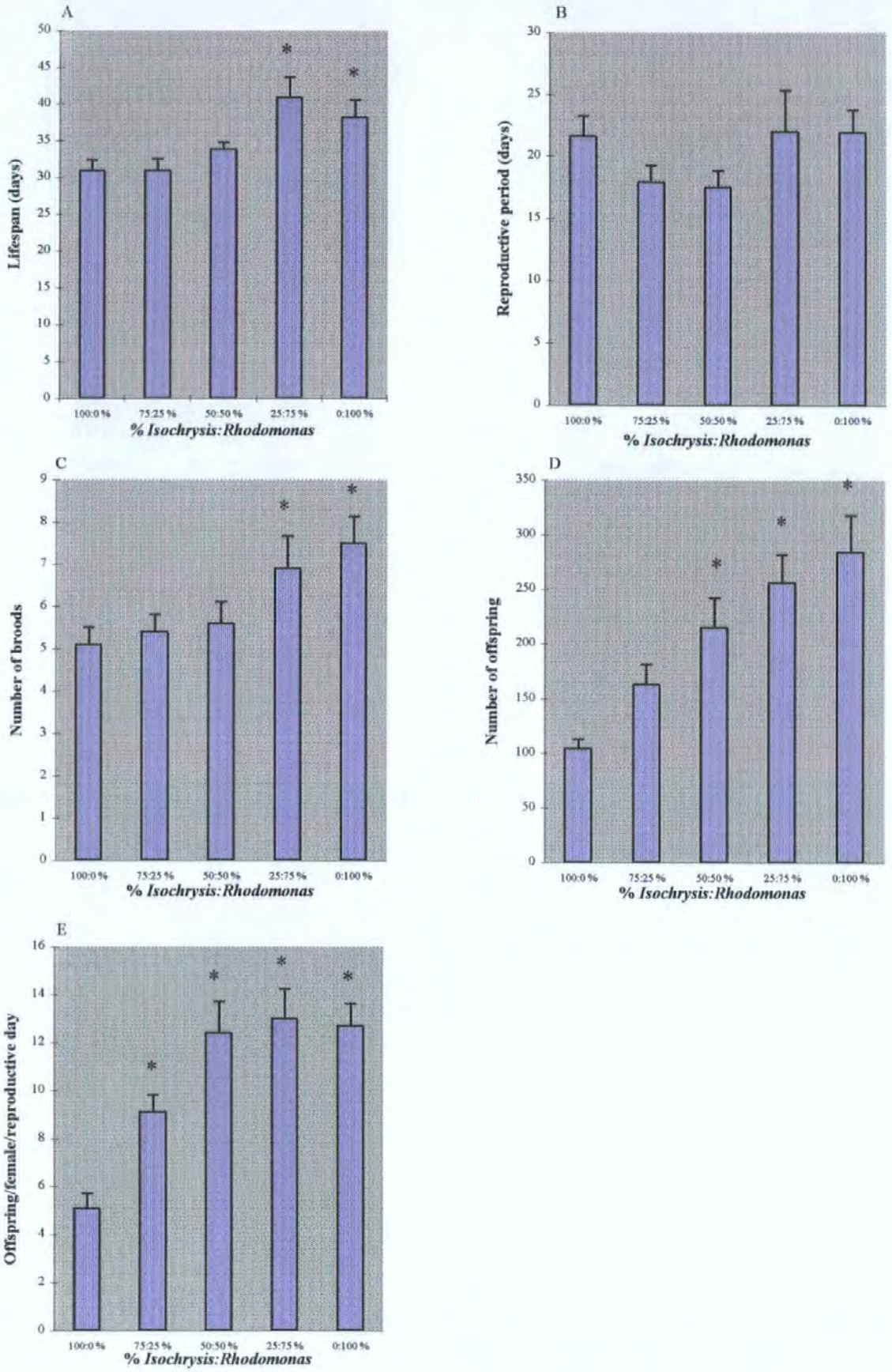


Figure 5.1. The effect of algal diet (different combinations of *Isochrysis* and *Rhodomonas* on (A) lifespan, (B) reproductive period, (C) total number of broods, (D) total number of offspring and (E) the number of offspring per female per reproductive day produced by *Tisbe battagliai.* Values are expressed as the mean ±1SE [* significant difference ($P < 0.05$) compared with 100:0 diet].

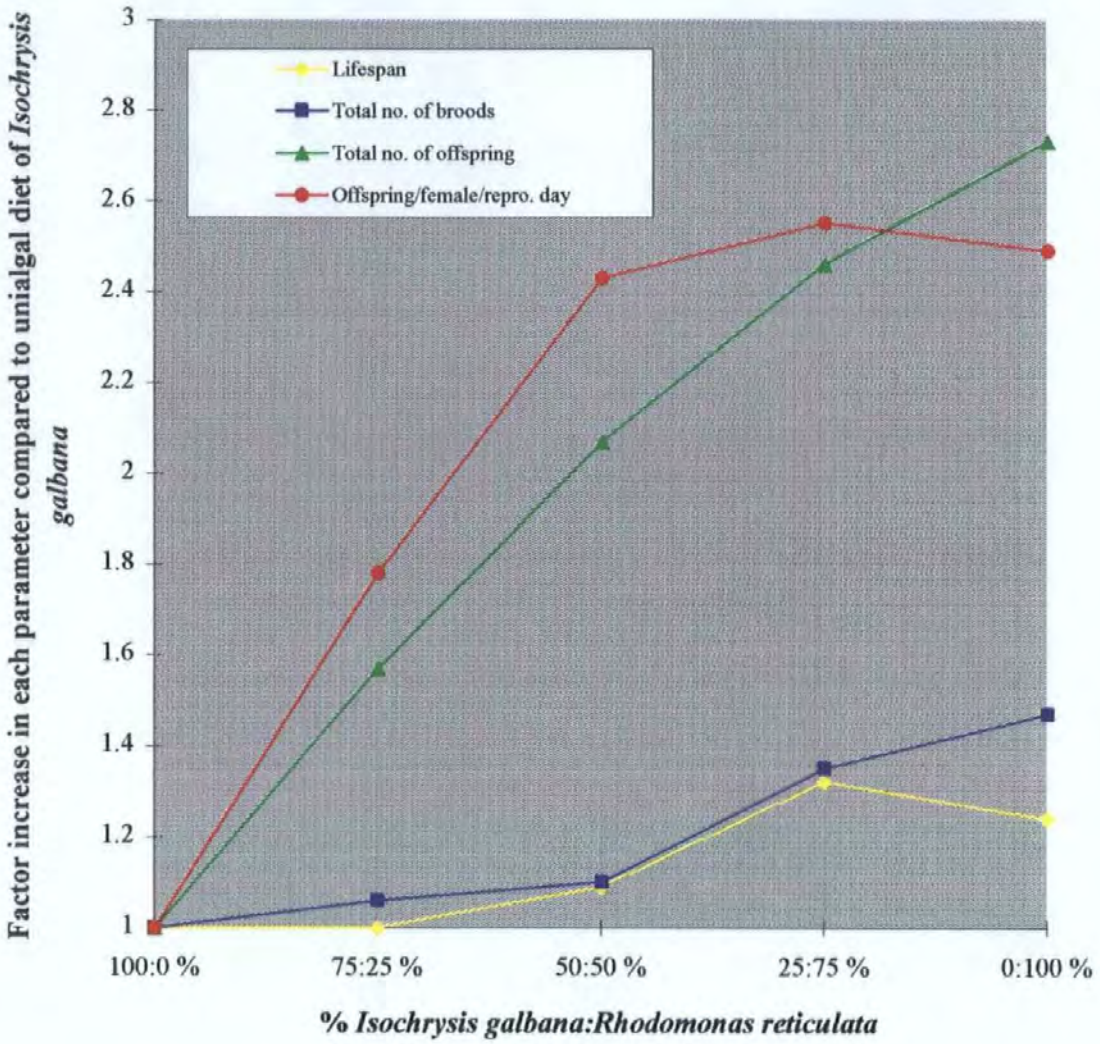


Figure 5.2. The effect of algal diet on the reproductive lifespan and reproductive parameters of *Tisbe battagliai*. Results represent the increase in each parameter compared with the unialgal diet of *I. galbana* (100: 0).

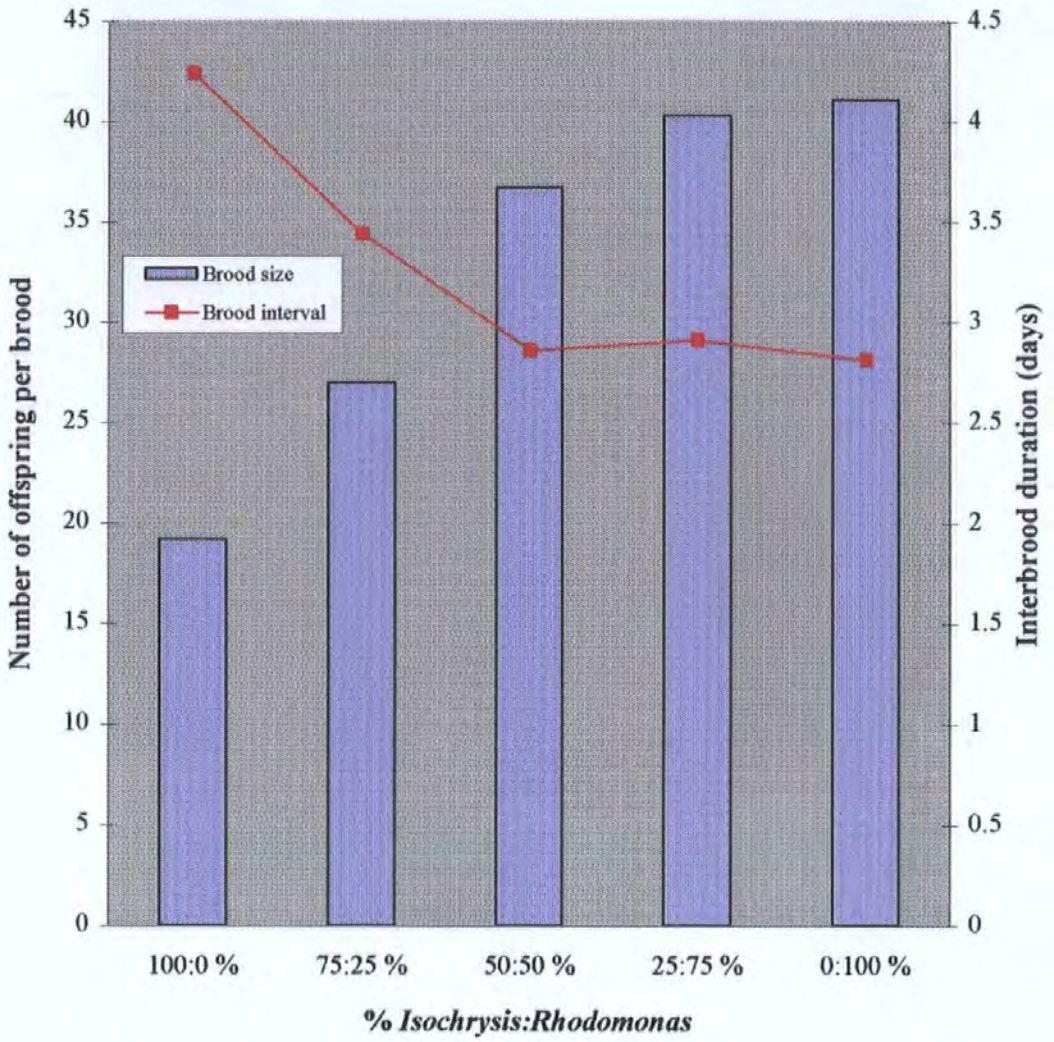


Figure 5.3. The effect of algal diet on brood size and interbrood duration in *Tisbe battagliai*. First broods were not produced under the influence of the test conditions and were excluded from the analysis. In some diets, copepods failed to produce more than 8 broods, therefore, the data represent overall mean values for broods 2 - 8.

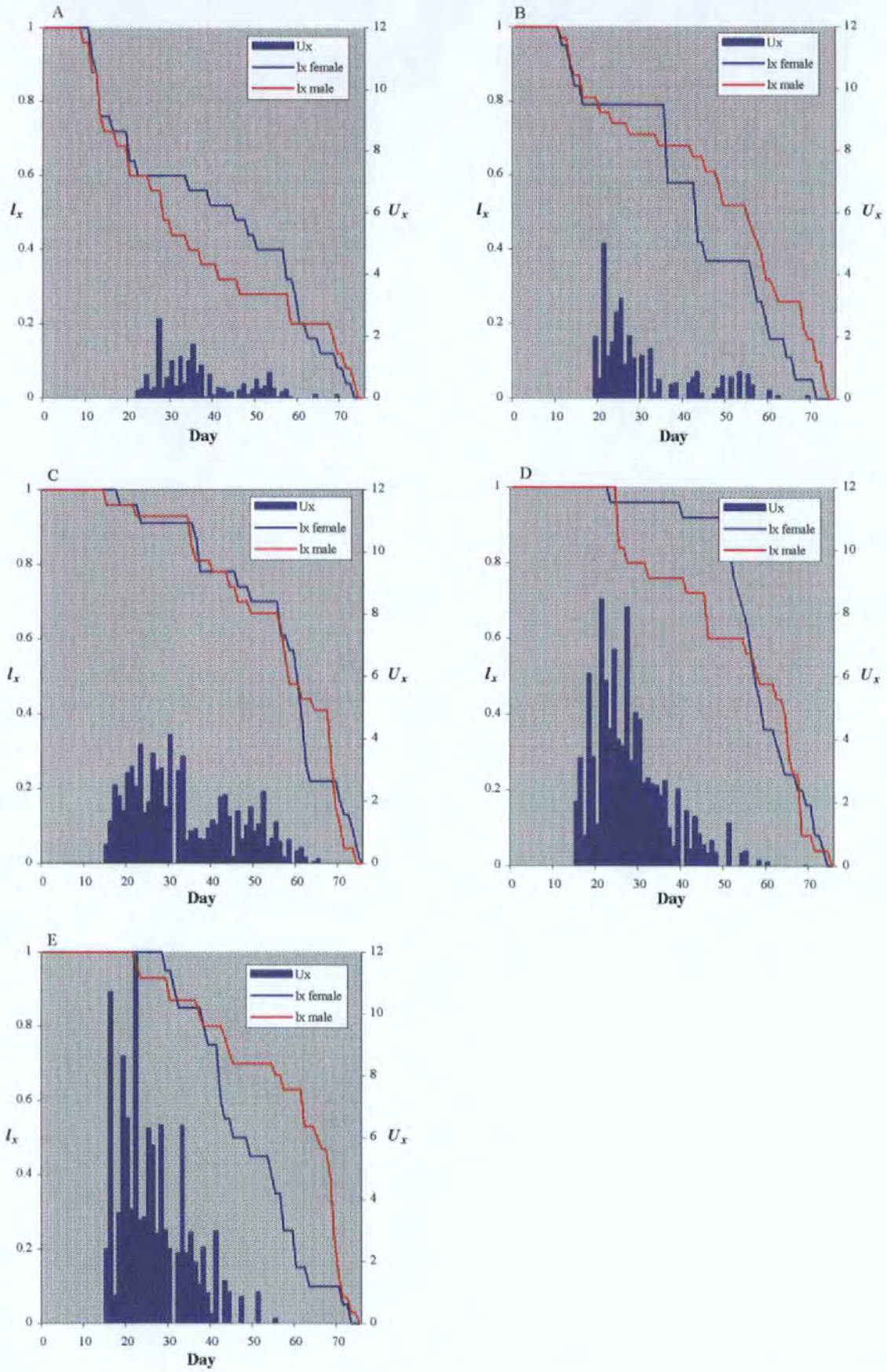


Figure 5.4. Age-specific survival (l_x) and fecundity (U_x) of *Tisbe battagliai* fed an algal diet composed of (A) 100:0%, (B)75:25%, (C) 50:50%, (D) 25:75% and (E) 0:100% *Isochrysis galbana*:*Rhodomonas reticulata*.

CHAPTER 6

THE EFFECT OF PENTACHLOROPHENOL ON THE POPULATION DYNAMICS OF *TISBE BATTAGLIAI*: MEASUREMENT OF EFFECTS ON POSTEMBRYONIC DEVELOPMENT, REPRODUCTION AND THE COMPLETE LIFE CYCLE

6.1. INTRODUCTION

The main aim of this research programme has been to develop test methods for evaluating the potential long-term effects of chemicals on the marine environment using *Tisbe battagliai*. An understanding of the basic biology of the test species was an essential requirement for the development of laboratory culture and toxicity test procedures and the evolution of test methods for *Tisbe battagliai* has proceeded alongside an investigation of the influence of key environmental variables on the population dynamics of this species (Chapters 2 - 5). In nature, pollutant effects are mediated by biotic and abiotic factors which can enhance or inhibit the impact of chemicals on organisms. Simple laboratory test systems do not adequately represent the fate and effect of chemicals in the natural environment and these factors must be considered when extrapolating from laboratory tests to the field situation (Forbes & Forbes, 1994). As an example, most laboratory investigations of the toxicity of chemicals to aquatic species are performed at single, usually unlimiting food concentrations, yet in the natural environment, the supply and associated nutritional value of food items changes frequently in time and space and is subject to seasonal availability. The provision of a nutritionally complete diet has important consequences for animal fitness and nutritional deficiencies may cause significant changes in the sensitivity of organisms to chemicals (Cowgill, 1986; Vanni & Lampert, 1992; Brett, 1993). Landis (1986) argued that a major drawback of chronic laboratory toxicity tests is that they do not measure reproductive rates as a function of resource availability, which he contended was a critical variable in the determination of the community-level impact of toxicants. Despite knowledge of the importance of environmental variables on pollutant toxicity, and its relevance to the field situation, relatively few studies have investigated the combined effects of environmental factors on the toxicity of chemicals (Persoone *et al.*, 1989). Knowledge of these interactions, and

more importantly, their incorporation into toxicity test procedures may improve the ability of laboratory tests to predict effects in the field.

The main objective for the following series of experiments was to investigate the potential interaction between temperature, food (quantity and quality) and the toxicity of chemicals to *Tisbe battagliai*. These experiments provide further information on the effect of food quantity and quality on the population dynamics of this species and help define the optimal diets for use in chronic toxicity test procedures. Studying the combined effect of environmental variables and chemical contaminants under laboratory conditions may significantly improve our understanding of how key environmental variables affect organisms and their response to toxicants under more environmentally realistic exposure situations.

6.2. MATERIALS AND METHODS

6.2.1. Copepods and algal culture

The procedures for culturing *Tisbe battagliai* are described fully in Chapter 2. Experimental cultures were maintained in filtered (0.2 μm), seawater (approximately 35‰) obtained from Tor Bay, Devon, and held in constant temperature rooms at 15, 20 and 25°C under a photoperiod of 16 h light:8 h dark and dawn:dusk transition periods of 20 mins. The procedures for culturing the algal species *Isochrysis galbana* and *Rhodomonas reticulata* are described fully in Chapters 2 and 5 respectively.

6.2.2. Test chemical

The test chemical, pentachlorophenol (PCP), was chosen because it is often used as a reference toxicant (Goodfellow & Rue, 1989), it has been shown to be relatively stable in test solutions within renewal periods of 48 h (Stephensen *et al.*, 1991), and its chemistry,

toxicology and fate in the environment have been reported widely (Wild *et al.*, 1992; Hobbs *et al.*, 1993). The chemical was obtained from Aldrich Chemicals and was stated to have a 99% purity. Stock solutions of PCP were prepared in triethylene glycol, stored in the dark and replaced weekly. Test concentrations were prepared by the addition of appropriate aliquots of stock solution in dilution water. Additions were made gradually by microlitre syringe whilst stirring by magnetic follower; solutions were stirred for 30 mins prior to use. The solvent control, and all test concentrations, contained 0.1 ml l^{-1} of triethylene glycol; the control solution consisted of dilution water only. Test solutions were not chemically analysed and all values of PCP quoted in this document are based on nominal concentrations.

6.2.3. Test procedures

Experiments were carried out in temperature-controlled rooms (± 1°C) with a photoperiod of 16 h light:8 h dark and dawn:dusk transition periods of 20 mins. The dilution water was the same as that used to culture *Tisbe battagliai* and consisted of filtered (0.2 µm), natural seawater (approximately 35‰) obtained from Tor Bay, Devon. Stock suspensions of *Isochrysis galbana* and *Rhodomonas reticulata* were diluted with filtered (0.2 µm) seawater to provide the appropriate algal food concentrations. Carbon values, and cell concentrations of the freshly prepared algal suspensions, were determined using a Dohrman DC-190 high temperature carbon analyser and a Model ZB Coulter Counter respectively. Algal suspensions were stored in a refrigerator in the dark and used within 7 days. Specific details of the individual experiments are described below and a full summary of the preliminary and definitive experiments undertaken is provided in Table 6.1.

6.2.3.1. Acute toxicity

Acute toxicity tests were conducted on adult ovigerous females and nauplii to evaluate potential differences in sensitivity between these two different life-history stages. Nauplii (±24 h) were obtained by isolating ovigerous females from a mass culture. Females with egg sacs were isolated 24 h before an experiment, so that nauplii collected the next day would be nearly the same age (±24 h). Adult females, at the start of their reproductive period, were obtained from a mass culture derived initially from a cohort of nauplii collected over 24 h. After approximately 11 days, females carrying their first egg sac were visible and these were selected as test animals. At each temperature, 96 h acute toxicity tests were initiated with 20 nauplii and 20 adult females at each concentration, five each in four test chambers (wells of tissue culture plates) containing 5 ml of test solution. Acute toxicity tests are performed usually without the addition of food, however, interpretation of the results may be difficult if the test animals are sensitive to food limitation during the exposure period. Furthermore, chronic toxicity experiments are conducted with feeding, therefore, comparisons between acute and chronic toxicity data are best made under the same conditions. Consequently, present tests were performed with and without the addition of algae as food. Tests were started by the random addition of copepods to test solutions after checks to ensure that the solutions were at the specified temperature. Mortality of *Tisbe* was assessed after 24, 48, 72 and 96 h. Mortality was defined as the absence of any movement by the copepod for a period of 15 secs when examined at a magnification of 25x for nauplii and 16x for adults. Dead copepods were removed at each observation point.

The test solutions were prepared on the day of use and renewed partially each day. On each renewal day, 80% (4 ml) of the test solution was replaced with freshly prepared test solution containing 0 (unfed) or 3250 μgC l^{-1} of *Isochrysis galbana*. The pH and dissolved oxygen concentration was measured in the newly prepared test solution and in the control

(dilution water used to prepare the test solutions). On each renewal day, the pH and dissolved oxygen concentration of the old test solutions were measured in each treatment. Temperatures were measured daily in an additional replicate chamber (without *Tisbe*) for each treatment.

6.2.3.2. Chronic toxicity: postembryonic development

Essentially, test procedures were the same as those described in Chapter 2 and the progress of individual nauplii was followed from hatching through to adulthood. Ten nauplii (< 5 h old) were exposed to different combinations of temperature, food concentration and PCP until the adult stage was reached. Individual nauplii were added randomly to 10 wells of tissue culture plates, each containing 2 ml of test solution. Observations of moulting and mortality were made at 24 h intervals and the surviving copepods were transferred to duplicate test chambers containing freshly prepared test solutions. In addition, body measurements were made after surviving copepods had reached the adult stage. Propylene phenoxytol (2-phenoxyethanol) was used as a relaxing agent (0.15%) prior to sacrificing the copepods with 3% buffered formalin. Body length (anterior end of the rostrum to the posterior edge of the last abdominal segment) of each adult was measured using a microscope with an eyepiece graticule previously calibrated with a stage micrometer. Temperature (daily), and the pH and dissolved oxygen concentration (at least weekly) was measured in each treatment.

6.2.3.3. Chronic toxicity: reproduction

Test procedures were generally the same as those described in Chapter 3. From the time of first reproduction, adult females were exposed to different combinations of temperature, food concentration and PCP for 14 or 21 days. Several broods of offspring were released by females within these exposure periods, thereby, enabling a realistic measure of offspring production to be made. Adult females were derived from a cohort of nauplii (< 24 h

old) reared in cultures maintained at the appropriate test temperature until the first egg sacs were visible. Individual females were added randomly to 10 wells of tissue culture plates, each containing 5 ml of test solution. Observations of mortality (defined as the absence of any movements by the organism, when examined by microscope for a period of 15 secs) and the total number of offspring produced by each female were made at 24 h intervals. Surviving copepods were transferred to duplicate test chambers containing freshly prepared test solutions and the appropriate concentration of algae. Temperature (daily), and the pH and dissolved oxygen concentration (at least weekly) was measured in each treatment. Experiments were started with ovigerous females derived from laboratory cultures, therefore, production of the first brood of offspring was judged to have been influenced by the previous culture regime. Consequently, analysis of reproductive parameters (total number of offspring produced) excluded first brood values.

6.2.3.4. Chronic toxicity: complete life cycle (life-table analysis)

In the previous experiments, the effect of PCP on the development and reproduction of *Tisbe battagliai* was measured independently. Separate experiments are, for reasons of biological and practical utility, easier to perform and require less time to carry out than complete life-cycle experiments, however, life-cycle exposures provide more biological information. In life-cycle experiments, the exposure period includes survival and development of the sensitive (critical) early life stages, development of the juvenile stages to reproductive maturity and measures events throughout the reproductive lifespan. Furthermore, the survival and fecundity data from complete life-cycle exposures can be used to compute population parameters such as the intrinsic rate of natural increase. The objective of this experiment was to determine the influence of food quantity and quality on the response of *T. battagliai* to prolonged (complete life cycle) exposure to sublethal concentrations of PCP. A further

objective was to determine whether the effects of PCP on development and reproduction, measured separately, are predictive of effects measured over the complete life cycle (hatching to death) of *T. battagliai*. The experimental design was constructed to allow complete life-cycle exposures using different concentrations of PCP (0, 10, 32 and 100 μg l^{-1}) and to provide a comparison between single and mixed species algal diets. The unialgal diet consisted of *Isochrysis galbana* and the mixed diet a 1:1 ratio (based on carbon) of *I. galbana* and *Rhodomonas reticulata*. The algal concentrations were selected to provide a comparison of biological response at unlimiting (3250 μgC l^{-1}) and limiting (1300 μgC l^{-1}) food concentrations. Experiments were conducted at a single temperature (20°C), selected on the basis of results from development and reproduction experiments which suggested that the development and reproduction of *T. battagliai* is more sensitive to PCP at 20°C than at 15 and 25°C (Sections 6.2.3.2 and 6.2.3.3).

Test procedures were based on those described in Chapter 4 but modified (see below) to reduce the amount of work required to service the relatively large experimental design. The experimental design consisted of two distinct stages. In the first stage, fifty nauplii (< 24 h old) were added randomly to crystallising dishes containing 200 ml^{-1} of test solution and maintained at each combination of food and PCP concentration until all females in the appropriate treatments had produced their first egg sac. At this time, the sex of each copepod, and the time taken for each female to produce their first egg sac, was noted. In the second stage, 10 individual females were selected randomly from the common pool of ovigerous females in each treatment and added individually to 10 wells of tissue culture plates, each containing 5 ml of test solution. At the same time, the remaining ovigerous females, unfertilised females and males from the original cohort of 50 individuals were discarded. If the experimental design had not been so large, all members of the cohort would have been exposed through to day at death. However, the size of the experimental design precluded measurement of all individuals hence

the use of a modified life table consisting of 10 females per treatment. Individual females were maintained at each concentration until death and the age-specific survival and fecundity schedules were used to compute the intrinsic rate of natural increase (r_m) according to the procedures described in Chapter 4.

Observations of mortality (defined as the absence of any movements by the organism, when examined by microscope for a period of 15 secs), the sex of each copepod and the time taken for each female to produce their first egg sac were recorded. Following transfer of individual females (n=10 after transfer), survival and the total number of offspring produced by each female were recorded at 24 h intervals. Surviving copepods were transferred to duplicate test vessels containing freshly prepared test solutions at the appropriate algal concentration. Temperature (daily), and the pH and dissolved oxygen concentration (at least weekly) was measured in each treatment.

6.2.4. Statistical analyses

In the acute toxicity experiments, the LC50 values were calculated at different time intervals by the moving average angle or binomial method (Stephan, 1977). Statistically significant effects on copepod survival during postembryonic development (Section 6.3.2.1) and reproduction (Section 6.3.2.2) were calculated using Fisher's Exact Test, a contingency table procedure (Finney *et al.*, 1963). The data for copepod development times and offspring production were tested for normality and equality of variances prior to analysis of variance techniques (Sokal & Rohlf, 1995). Data that failed to meet the assumptions for analysis of variance were analysed using non-parametric (distribution-free) techniques (Steel, 1959; Hollander & Wolfe, 1973). Using the data from definitive experiments for postembryonic development and reproduction (Table 6.1), the interaction of temperature, food concentration and PCP concentration on copepod development times and offspring production was tested

using 3-way ANOVA, using a 3 (temperature) x 3 (food concentration) x 4 (PCP concentration) factorial design. Similarly, potential interactions between algal diet and PCP concentration were examined using the reproduction data from complete life-cycle experiments (Table 6.1) in a 2-way ANOVA, using a 4 (diet) x 4 (PCP concentration) factorial design. The sex ratio of adult copepods was examined using a contingency table procedure (Hayslett & Murphy, 1976) to identify significant departures from a sex ratio of 1:1. Pairwise comparisons between male and female body length (control data only) and time to first egg sac at corresponding diet (unialgal versus mixed species algal diet) and algal concentration (1300 versus 3250 $\mu gC\ l^{-1}$) were made by Student's *t*-test (one-tailed).

6.3. RESULTS

6.3.1. Acute toxicity

The median lethal concentration (LC50) values for the naupliar and adult female life-history stages decreased with increasing length of exposure duration (Table 6.2). Comparison of the 96 h LC50 values between the naupliar and adult female life-history stages, with and without the addition of algal food, are shown in Figure 6.1. Differences in 96 h LC50 values could not be statistically evaluated but trends were identified based on the confidence interval data. The 96 h LC50 values for both fed and unfed adult females ranged from 403 - 541 $\mu g\ l^{-1}$ at 15 and 25°C. By comparison, the corresponding 96 h LC50 values at 20°C were lower (338 and 294 $\mu g\ l^{-1}$), suggesting a potential increase in sensitivity for adult females at this temperature (Fig. 6.1A). A similar trend was observed for the naupliar stage although the differences were small and probably not significant. The 96 h LC50 values for nauplius and adult female were lower for unfed versus fed copepods (Fig. 6.1B) but differences were

probably not significant except for the naupliar stage exposed at 25°C (LC50 values of 1023 and 466 μg l^{-1} for fed and unfed nauplii respectively).

6.3.2. Chronic toxicity: preliminary experiments

The results from these acute toxicity tests were used to define a range of test concentrations for preliminary chronic toxicity experiments. The objective of these next experiments was to define the effect of PCP on the postembryonic development and reproduction of *Tisbe battagliai*.

6.3.2.1. Postembryonic development

6.3.2.1.1. Effects of pentachlorophenol

Experiments were carried out at temperatures of 15 and 20°C, and PCP concentrations up to and including 1000 μg l^{-1} (Table 6.1). Significant copepod mortality (≥ 40%) was observed in treatments containing 56 - 1000 μg l^{-1} PCP and effects were most pronounced at the lowest food concentration (520 μgC l^{-1}) and at 20°C (Fig. 6.2). At 20°C, mortality increased with successive reductions in algal concentration from 3250 - 520 μgC l^{-1} and, at the lowest algal concentration (520 μgC l^{-1}), significant mortality (70 - 100%) was observed in all PCP treatments over the range 56 - 1000 μg l^{-1}.

The time taken for surviving male and female copepods in each treatment to reach the adult stage was measured although no attempt was made to statistically analyse these data due to the relatively small number of surviving copepods at PCP concentrations ≥ 100 μg l^{-1}. Although the small numbers of surviving copepods precluded statistical analysis, visual comparison of the adult development times at 15 and 20°C for surviving copepods in the controls and PCP concentrations up to and including 100 μg l^{-1} were made (Figs 6.3 and 6.4

respectively). The data suggest a potential increase in adult development times at 100 μg l^{-1} PCP, at low food concentrations. For example, differences in adult development times (data for both sexes combined) between copepods reared in the solvent control and 100 μg l^{-1} PCP treatments amounted to 0.33 - 0.40 d at algal concentrations of 3250 μgC l^{-1} and 1.0 - 1.2 d at 1300 μgC l^{-1}. At 520 μgC l^{-1} (the lowest algal concentration tested), differences amounted to 1.7 d at 15°C but comparisons could not be made at 20°C as 90% of the copepods died before reaching the adult stage at 100 μg l^{-1} PCP.

6.3.2.1.2. Effect of food concentration

The time taken for male and female copepods to reach the adult stage was dependant on food concentration (Fig. 6.5). Due to the relatively small number of copepods used in the experimental design, statistical evaluation of differences in male and female development times proceeded using pooled control and solvent control data. With increasing food concentration, individual variability in male and female adult development times became smaller and, as a result, relatively small differences in development times between male and female were found to be statistically significant. At 15°C, females took significantly longer than males to reach the adult stage at all algal concentrations (520, 1300 and 3250 μgC l^{-1}) and these differences amounted to 3.5, 1.05 and 0.58 d respectively (Fig. 6.5). At 20°C, females took significantly longer than males to reach the adult stage at 520 and 1300 μgC l^{-1} (7.6 and 0.56 d respectively) but not at 3250 μgC l^{-1} (Fig. 6.5). These results have implications for the development of toxicity test methods based on postembryonic development; at suboptimal food concentrations, the experimental design must allow for differences in development times between the sexes. Statistical analysis of these data also revealed a significant reduction [ANOVA followed by Bonferroni's *t* test (one-tailed) or Wilcoxon's Rank Sum Test; $P < 0.05$] in development time for both sexes following successive increases in algal concentration.

6.3.2.2. Reproduction

Preliminary experiments were carried out at temperatures of 15 and 20°C, and copepods were exposed to PCP concentrations ≤ 1000 μg l^{-1} for 21 days (Table 6.1). The total numbers of offspring produced by each female in the different treatments are shown in Figure 6.6.

At 15°C, there was a significant reduction (Fisher's Exact Test, $P < 0.05$) in copepod survival at PCP concentrations ≥ 180 μg l^{-1}. Results were dependent upon algal concentration and, after 21 days, significant mortality (Fisher's Exact Test, $P < 0.05$) was observed in PCP treatments receiving algal concentrations of 1300 and 3250 μgC l^{-1} but not 520 μgC l^{-1}. Few offspring were produced at algal concentrations of 520 and 1300 μgC l^{-1} (e.g. 4.6 and 10.7 offspring per female respectively in the solvent control) and these numbers were insufficient for statistical analysis. At 3250 μgC l^{-1}, there was no significant difference in numbers of offspring produced in the control and solvent control treatments. Compared with the solvent control, there was a significant reduction in offspring production at PCP concentrations ≥ 32 μg l^{-1}, therefore, the no observed effect concentration (NOEC) for reproduction at 15°C was 18 μg l^{-1} PCP (Fig. 6.6A)

At 20°C, mortality of the copepods (60 - 100%) after 21 days was significant (Fisher's Exact Test, $P < 0.05$) in all control and PCP treatments receiving algal concentrations of 520 μgC l^{-1} and only a few offspring were produced (e.g. 9.4 offspring per female in the solvent control) (Fig. 6.6B). At higher algal food concentrations (1300 and 3250 μgC l^{-1}), copepod mortality after 21 days was significantly increased (Fisher's Exact Test, $P < 0.05$) at PCP concentrations ≥ 320 μg l^{-1} compared with the controls. Differences in offspring production between the control and solvent control treatments were not significant and the mean numbers of offspring produced by each female in the control and solvent control treatments ranged from 137 - 148 at 1300 μgC l^{-1} and 165 - 208 at 3250 μgC l^{-1} (Fig. 6.6B). Compared with the

solvent control, there was a significant reduction in offspring production at PCP concentrations ≥ 180 μg l^{-1} at 1300 μgC l^{-1} and ≥ 320 μg l^{-1} at 3250 μgC l^{-1} (Fig. 6.6B). Therefore, the no observed effect concentrations (NOECs) for reproduction at algal concentrations of 1300 and 3250 μgC l^{-1} were 100 and 180 μg l^{-1} PCP respectively.

6.3.3. Chronic toxicity: definitive experiments

The results from the preliminary chronic experiments were used to define the experimental design for the next series of experiments. The objective of these experiments was to investigate the potential interaction between toxicant (PCP), temperature and food concentration on the population dynamics of *Tisbe battagliai.*

The experimental design for these experiments is summarised in Table 6.1. Experiments were performed at three temperatures (15, 20 and 25°C), three food concentrations (1300, 2055 and 3250 μgC l^{-1}) and four concentrations of PCP, including a solvent control (0, 10, 32 and 100 μg l^{-1}). Previous experiments (Section 6.3.2.2) have established that reproduction was very sensitive to reductions in food concentration and few offspring were produced at the lowest algal concentration of 520 μgC l^{-1}. Consequently, the lower limit of algal concentration in these current experiments was increased from 520 to 1300 μgC l^{-1} to provide measurable values for offspring production. The objective of these experiments was to investigate potential interactions between toxicant and environmental parameters on the development and reproduction of *Tisbe battagliai.* Therefore, the range of PCP concentrations was selected carefully to provide measurements of sublethal effect (development and reproduction), not mortality. Based on the results of preliminary experiments (Section 6.3.2), the following PCP concentrations were selected for the definitive experiments; 0 (solvent control), 10, 32 and 100 μg l^{-1}. The PCP concentrations of 10 and 100

$\mu g\ l^{-1}$ are within a factor of 34 - 60 (10 $\mu g\ l^{-1}$) and 3.4 - 6.2 (100 $\mu g\ l^{-1}$) of the 96 h LC50 value for the adult female (Table 6.2).

6.3.3.1. Postembryonic development

Temperature and PCP concentration, both independently and in combination, had significant effects on the development to the adult stage (Table 6.3). The lack of interaction between temperature and algae is in agreement with the results from previous experiments (Chapter 2) and confirm that these variables act independently on postembryonic development. The algal concentrations used in these experiments were not expected to influence copepod development times as the results from previous experiments (Chapter 2) indicated no differences in development times between copepods fed 1300 and 3250 $\mu gC\ l^{-1}$.

The development time (days) from hatching to the adult stage (N1-A) for *Tisbe battagliai* exposed to different concentrations of PCP (0, 10, 32 and 100 $\mu g\ l^{-1}$), algal food (1300, 2055 and 3250 $\mu gC\ l^{-1}$) and temperatures of 15, 20 and 25°C are summarised in Tables 6.4 - 6.6 respectively. Further analysis was carried out to determine the effect of the independent variables PCP and algal concentration on adult development times. Statistical analysis of the development data was performed for both sexes combined. No attempt was made to analyse the data for individual sexes due to the very small numbers of copepods involved. Experimental approaches to measure the development times for male and female copepods would require much higher numbers of test organisms than used here (n = 10).

6.3.3.1.1. Effect of pentachlorophenol

At 15°C, statistical analysis of the data for surviving copepods (both sexes combined) revealed no significant differences in the development times between copepods in the solvent control and PCP treatments (Table 6.4). At 20°C, statistical analysis of the data for surviving

copepods (both sexes combined) revealed significant differences in the development times between copepods in the solvent control and PCP treatments, but only for those treatments receiving the lowest food concentration of 1300 μgC l^{-1} (Table 6.5). Compared with the solvent control, copepods exposed to PCP concentrations of 32 and 100 μgC l^{-1} took significantly longer to reach the adult stage at 1300 μgC l^{-1} (Table 6.5). At 25°C, all copepods reached the adult stage and there were no significant differences in the development times between the solvent control and PCP treatments (Table 6.6).

6.3.3.1.2. Effect of food concentration

Analysis of the solvent control data at each test temperature (15, 20 and 25°C), revealed no significant differences (1-way ANOVA) in adult development times (both sexes combined) between the algal concentrations of 520, 1300 and 3250 μgC l^{-1} (Tables 6.4 - 6.6). However, combining the data for both sexes, which was necessary to provide sufficient data for analysis, may have masked more subtle differences between the responses of male and females to the different food regimes. These concerns are justified as preliminary experiments (Section 6.3.2.1.2) identified significant differences between male and female copepods in the time taken to reach the adult stage at algal concentrations of 520 and 1300 μgC l^{-1}. Similar concerns apply to the use of combined sex data to analyse the potential effects of PCP on development times; it is possible that male and female copepods respond differently to PCP.

The body size (length) of those copepods that successfully reached the adult stage was measured to confirm visual observations that females are larger than males. Sexual dimorphism in size is well established within the Harpacticoida with, in the majority of species, females tending to be larger than males (Hicks & Coull, 1983). Results for *Tisbe battagliai* show that females are larger than males and the body length of control animals ranged from 628 - 670 μm for females and 538 - 557 μm for males, depending on test temperature (Table

6.7). No attempt was made to statistically analyse the data due to the relatively small number of individual male and female copepods available for comparison. Due to sexual dimorphism in the size of *T. battagliai*, experimental approaches using body size as a measurement parameter would, for reasons of improved statistical analysis, require much higher numbers of copepods than used here (n = 10).

6.3.3.2. Reproduction

Temperature and algal concentration had significant independent and synergistic effects on offspring production, and PCP had similar significant effects on offspring production in combination with temperature but not with algae (Table 6.3).

6.3.3.2.1. Effect of pentachlorophenol

The effect of PCP on the total numbers of offspring produced by *Tisbe battagliai* after 14 days exposure to different temperature (15, 20 and 25°C) and food concentrations (1300, 2055 and 3250 $\mu gC\ l^{-1}$) is shown in Figure 6.7. Significant differences in offspring production between copepods in the control and PCP treatments were observed at 20°C but not at 15 and 25°C. Effects were dependent upon food concentration and, at the lowest algal concentration tested (1300 $\mu gC\ l^{-1}$), there was a significant reduction in the numbers of offspring produced at PCP concentrations of 32 and 100 $\mu g\ l^{-1}$ (Fig. 6.7) Effects of PCP were less pronounced at higher algal concentrations and, at 2055 $\mu gC\ l^{-1}$, a significant reduction in offspring production was observed only at 100 $\mu g\ l^{-1}$; significant differences were not detected at the highest algal concentration tested (3250 $\mu gC\ l^{-1}$) (Fig. 6.7).

6.3.3.2.2. Effect of food concentration

Statistical analysis of the control data at each temperature identified a significant reduction (1-way ANOVA, $P < 0.05$) in the numbers of offspring produced by copepods fed 1300 µgC l^{-1} compared with those at 2055 and 3250 µgC l^{-1}; differences in offspring production between 2055 and 3250 µgC l^{-1} were not significant (Fig. 6.7).

6.3.3.3. Complete life cycle

Experiments were performed with mixed species (*Isochrysis galbana* and *Rhodomonas reticulata*) and unialgal (*I. galbana*) diets and, for convenience, the description of results proceeds without further reference to the algal species. Juvenile survival on the different algal diets was very high (92 - 100%) except for the low ration (1300 µgC l^{-1}) unialgal diet where a proportion (6 - 24%) of the copepods in the control and PCP treatments died before reaching the adult stage (Table 6.8). The experimental design consisted of sixteen different treatments (4 diets, each containing 4 treatments) and, with the exception of one treatment in the low ration unialgal diet, the sex ratio of survivors in each cohort did not depart significantly from 1:1 (Table 6.8).

Within each of the four algal diets tested, differences in the time to production of the first egg sac between surviving females in the control and those exposed to PCP concentrations up to and including 100 µg l^{-1} were not significant (Table 6.9). There were, however, significant effects of algal concentration and diet composition on the time to production of the first egg sac (Table 6.9). For the unialgal and mixed species algal diets, a reduction in algal concentration from 3250 to 1300 µgC l^{-1} (2.5 times less algae) resulted in a significant increase in the time to production of first egg sac (Table 6.9). For example in the control, a reduction in algal concentration from 3250 to 1300 µgC l^{-1} extended the time to production of the first egg sac by 2.4 days for copepods fed a mixed algal diet and by 6.2 days for those fed a unialgal

diet (Fig. 6.8). At low and high ration algal concentrations (1300 or 3250 µgC l^{-1}), the production of the first egg sac was significantly delayed when copepods were fed on unialgal rather than mixed species algal diets (Table 6.9). For example, control females produced their first egg sac significantly faster on a mixed species rather than a unialgal diet and these differences amounted to 1.3 days at 3250 µgC l^{-1} and 5.1 days at 1300 µgC l^{-1}. At this stage (i.e. production of the first egg sac by the female) of the life-cycle experiments, 10 ovigerous females from each treatment (fewer if insufficient number were produced) were isolated randomly from a common pool of females and exposed individually to the same treatments (4 diets, each containing 4 treatments) through to day at death. The remaining copepods were discarded. Life-table calculations, and the computation of population parameters such as the intrinsic rate of natural increase r_m, should in theory, proceed with all (female) members of the cohort. However, to do so on this occasion would, for practical reasons, have been impossible with the chosen experimental design. Consequently, only 10 females per treatment were selected for the complete life-cycle exposures.

A summary of the results from complete life-cycle exposures with female *Tisbe battagliai* at the four different algal diets is shown in Tables 6.10 - 6.13. Trends in key reproductive parameters such as the total numbers of offspring produced were compared with computed values of r_m derived from the survival and fecundity data. Algal diet and PCP concentration, both independently and in combination, had significant effects on offspring production (Table 6.14). At the high ration (3250 µgC l^{-1}) unialgal diet, differences in reproductive parameters between the control and the highest concentration of PCP (100 µg l^{-1}) were not significant and values of r_m (0.238 and 0.216) were similar (Table 6.11). At the high ration mixed species algal diet, there were significant reductions in reproductive parameters (reproductive period, numbers of broods and numbers of offspring) at PCP concentrations of 10 and 100 µg l^{-1} compared with the control, however, values of r_m (0.257 - 0.270) remained

within a relatively narrow range (Table 6.10). Greater reductions in r_m due to PCP were found in treatments receiving the low algal ration of 1300 µgC l^{-1} (Fig. 6.9B). Treatments receiving 1300 µgC l^{-1} of a mixed species or unialgal diet showed significant reductions in lifespan and reproductive parameters (reproductive period, numbers of broods) at PCP concentrations of 32 and 100 µg l^{-1} compared with the control (Tables 6.12 and 6.13). Within-treatment variability in offspring production between individual females was relatively high at low algal concentrations (1300 µgC l^{-1}) and this may explain why significant differences in offspring production between the control and PCP treatments (32 and 100 µg l^{-1}) were not detected. Compared with the control, reductions in r_m were most pronounced at 100 µg l^{-1} PCP in treatments receiving the mixed algal diet, and at 32 and 100 µg l^{-1} PCP in treatments receiving the unialgal diet (Fig. 6.9B). For those copepods fed with a unialgal diet of 1300 µgC l^{-1}, the life table (constructed with 10 fertile females) ignored effects on juvenile survival and female fertility, therefore, r_m may have been underestimated. The statistical significance of these reductions in r_m (19 - 28%) could not be established, however, differences of this magnitude may be significant for the population dynamics.

Higher values of r_m were achieved on mixed species rather than unialgal diets. For the control data, the magnitude of difference in r_m between unialgal and mixed algal diets was more pronounced at 1300 µgC l^{-1} (33%) than at 3250 µgC l^{-1} (12%) (Fig. 6.9A). In relation to food quantity, a reduction in algal concentration from 3250 to 1300 µgC l^{-1} resulted in a decrease in r_m (Fig. 6.9A). Results from the control treatments revealed that the magnitude of these differences was higher in the unialgal diet (45%) than it was for the mixed species algal diet (28%) (Fig. 6.9A).

6.4. DISCUSSION

Temperature, food and PCP (maximum concentration of 100 μg l^{-1}) had significant effects on the population dynamics of *Tisbe battagliai*. Temperature and PCP had a synergistic effect on postembryonic development and reproduction, and these effects of PCP were significant at 20°C but not at 15 and 25°C. The effects of PCP were dependent upon food concentration. Significant increases in the time taken to reach the adult stage, and significant reductions in offspring production, were observed at 32 and 100 μg l^{-1} PCP for copepods receiving the low (1300 μgC l^{-1}) but not the high (3250 μgC l^{-1}) ration algal diet. The results from life-table analysis were consistent with those described above for postembryonic development and reproduction. The effects of PCP on r_m were most pronounced at the low ration (1300 μgC l^{-1}) and at PCP concentrations of 32 and 100 μg l^{-1}. These findings raise some important questions concerning current approaches to regulatory ecotoxicology and their implications for the development and application of toxicity test methods are discussed below

Influence of environmental variables on the chronic toxicity of pentachlorophenol

Temperature effects on survival appeared to be more pronounced at 20°C than at 15 and 25°C. A similar temperature trend of PCP toxicity was observed in the chronic experiments. Published data on the influence of temperature on the acute toxicity of chemicals to invertebrates indicate there is a general increase in sensitivity with increasing temperature over the range 10 - 20°C (Persoone *et al.*, 1989) but responses may be chemical specific. For example, Cowgill *et al.* (1985) found no significant differences in the sensitivity of two freshwater crustaceans (*Daphnia magna* and *Ceriodaphnia dubia*) to three organic chemicals at 20 and 24°C but one chemical (chlorobenzene) was less toxic at the higher temperature.

Published data on the influence of temperature on the toxicity of PCP to marine invertebrates is limited but Tjeerdema *et al.* (1993) reported that an increase in water temperature from 9 to 19°C hastened the onset and recovery from the sublethal metabolic effects of PCP in the red abalone (*Haliotis rufescens*). The authors attributed these effects to a possible increase in general metabolic activity in the abalone at high temperatures, thus enhancing uptake of the biocide.

Results from the life-table experiments revealed that PCP toxicity was influenced both by food concentration and food quality (unialgal or mixed species algal diets). At the high ration (3250 μgC l^{-1}), which is representative of standardised chronic toxicity tests, PCP had no effect on the postembryonic development and reproduction of *Tisbe battagliai* at the maximum concentration tested (100 μg l^{-1}). By comparison, copepods fed 1300 μgC l^{-1} (2.5 times less algae) produced significantly fewer offspring and displayed a reduction in r_m at 32 and 100 μg l^{-1} PCP compared with the control. Therefore, the acute to chronic ratio (96 h LC50 for adult females/chronic NOEC) increased from approximately 3 at the high ration to 34 at the lower ration, a difference of one order of magnitude. These results suggest that the use of high ration diets in chronic toxicity experiments may underestimate the effect of chemicals on organisms exposed to suboptimal food concentrations, conditions that may be more prevalent in nature.

Differences in the life-table responses of copepods fed on unialgal and mixed species algal diets were more pronounced at the low algal ration. Although both groups of copepods received the same total concentration of algal carbon, the demographic parameters were consistently higher for those receiving mixed algal diets (*Isochrysis galbana* and *Rhodomonas reticulata*) instead of the unialgal diet (*I. galbana*). The mixed algal diet may provide a more nutritionally complete food for *Tisbe*, however, food quality may depend on the choice of algal species. For example, previous experiments have shown improved reproductive output by *T.*

battagliai on a unialgal diet of *R. reticulata* instead of *I galbana* (Chapter 5). Martinez-Jeronimo *et al.* (1994) investigated the effect of food type and concentration on survival, longevity and reproduction of *Daphnia magna*. These authors reported that the lowest food concentration of either algal diet was related to the lowest fecundities but there was a significant improvement in total offspring production and clutch size for *Daphnia* fed on a unialgal diet of *Scenedesmus incrassatulus* instead of *Ankistrodesmus falcatus*. These results suggest that the nutritional quality of the food (i.e. the type of diet) is important and changes in food quality may have a significant influence on the results of toxicity tests. Overall, the results from the *Tisbe* experiments confirm the potential benefits derived from feeding copepods on laboratory diets consisting of mixed rather than unialgal diets. Results also emphasise the importance of food quality in the response of organisms to both environmental variables and toxicants, indicating that aspects of both food quantity and quality must be considered in the development of laboratory culture methods and chronic toxicity test procedures.

Information on the acute and chronic toxicity of PCP to marine invertebrates is limited and comparisons should be made with caution as toxicity can be influenced by both pH and temperature (Hedtke *et al.*, 1986). Most of the reported LC/EC50 values are between 0.1 and 1.0 mg l^{-1} (Hobbs *et al.*, 1993). More recently, Smith *et al.* (1994) reported a 24 h LC50 value of 0.45 mg l^{-1} PCP for the 6 day old copepodid stage of *Tisbe battagliai*, and results indicated that the toxicity of chlorophenols to *Tisbe* conforms to those chemicals which have been characterised as 'polar' narcotics. In the current experiments, the acute toxicity of the copepodid stage was not measured, however, the 96 h LC50 values for PCP to *T. battagliai* (20°C) ranged from 394 - 592 µg l^{-1} for the nauplius and 294 - 338 µg l^{-1} for the adult female. Little information is available for chronic toxicity but Bengtsson & Bergstrom (1987) exposed fertilised females of the harpacticoid *Nitocra spinipes* to PCP for 13 days at 20 - 22°C and 7‰

salinity. The EC50 values based on fecundity (the number of live offspring produced) were calculated as 20 - 40 μg l^{-1} compared with a 96 h LC50 of 70 μg l^{-1}. The only other published sublethal toxicity data for PCP are for juvenile (8 d old) mysid shrimp *Americamysis bahia* at 25°C and 30‰ (Goodfellow & Rue, 1989) These authors reported 7 d chronic values [expressed as the geometric mean of the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) values] of 298 - 702 μg l^{-1} (mean of 498) based on the measurement of growth. By comparison, the effects of PCP on *T. battagliai* were most pronounced at 20°C and the chronic values were > 100 μg l^{-1} at the high ration algal diet and 18 μg l^{-1} [geometric mean of 10 (NOEC) and 32 (LOEC)] for the low ration algal diet.

Significance of these findings for current chronic toxicity test approaches

In laboratory toxicity tests, measurement of chronic toxicity does not usually take account of potential interactions between the toxicant and environmental variables, nor the subsequent effects on the response of the test organism. The results from present experiments demonstrate clearly that such interactions do occur and that they can influence significantly the toxicity of chemicals. Furthermore, laboratory chronic toxicity tests are performed in conditions of excess food but, in the natural environment, the seasonal availability of food resources, and their associated nutritional quality, may expose organisms to conditions of food limitation. Consequently, the extrapolation of results from laboratory studies conducted at near optimal food concentrations may underestimate the effects at the exposure conditions that are more likely to occur in the natural environment. Published data on the potential interactions between environmental variables and chemicals are limited but results from laboratory experiments provide evidence to support the general trends described here. Enserink *et al.* (1995) reported interactions between lead and the maturation process (size and age at egg deposition) in the freshwater crustacean *Daphnia magna*, especially at low food

concentrations. Similar interactions between effects of metals on maturation and the quantity or quality of food for cladocerans have been reported by other authors. Chandini (1989) observed interactions between cadmium and food concentration on age and size at first reproduction in *D. carinata* and these effects were more pronounced at low food levels. Korvisto *et al.* (1992) found that copper was more toxic at low food levels for five species of cladocerans in long-term (21 day) experiments. Persoone *et al.* (1989) examined the influence of environmental variables (temperature, salinity and water hardness) on the sensitivity of the rotifer *Brachionus plicatilis*, the brine shrimp *Artemia salina* and the freshwater crustacean *D. magna* to inorganic and organic chemicals. The latter authors reported significant interactions with chemicals for temperature-salinity and temperature-water hardness combinations for the three test organisms. For these variables (temperature-salinity and temperature-water hardness), the divergence in 24 h LC/EC50 values between standard test conditions and the various factorial combinations ranged from a factor of 2 to 27, changes were chemical and species specific, and caused both an increase and decrease in toxicity.

Although food availability can be considered as an environmental variable, it influences toxicity largely through its effect on the organism's nutritional status. When food availability is low, starvation and nutritional deficiencies can cause substantial changes in the sensitivity of an organism to chemicals. Cooney *et al.* (1983) reported an increase in sensitivity of the copepod *Diaptomus clavipes* to acridine as the food supply declined. Cowgill (1986) examined the critical variables for the successful culture and toxicity testing of cladocerans, and found that diet had a significant influence on the results of toxicity experiments. The data examined by Cowgill (1986) suggest that algal-fed cladocerans are less sensitive, and exhibit more consistent responses to toxicants, than those fed on diets consisting of trout chow and other supplements. In chronic toxicity tests (21 days in duration), *Daphnia magna* fed vitamin-enriched algae were less sensitive (by a factor of 2) than animals fed a diet consisting of trout

chow and cerophyll (Cowgill, 1986). The acute toxicity of sodium chloride to *D. magna* was 1661 mg l^{-1} for populations maintained on trout chow and alfalfa compared to 4571 mg l^{-1} for populations fed the green alga *Selenastrum capricornutum* (Cowgill, 1986). Also, Keating and Dagbusan (1986) reported differences in the acute toxicity of sodium chloride depending on whether *D. magna* was fed on a unialgal diet (*Chlamydomonas reinhardti*) (LC50 2250 mg l^{-1}) or a mixed species algal diet (*C. reinhardti*, *Ankistrodesmus convolutus* and *Nitzschia frustulum*) (LC50 3500 mg l^{-1}). Changes in food quantity and nutritional quality were found to have the largest effect on juvenile production in *D. magna* when compared to effects of light intensity, photoperiod and temperature (Lee *et al.*, 1986); at low food levels, effects of the other environmental variables became more important. Naylor *et al.* (1993) noted that differences in the fecundity of *D. magna* were correlated with the combination of algal species used in the diet. Brett (1993) measured the effect of high and low quality algal food, defined by total lipid and fatty acid content, on the demographic parameters of *Daphnia longispina* using life-table analysis; all demographic parameters improved when offspring and adults were fed on the high quality algal food. Kluttgen *et al.* (1996) reported that the response of *Daphnia magna* to 3,4-dichloroaniline was more pronounced at low food levels and increases in age at first egg production and reductions in offspring production occurred at all concentrations if the food level was low. Life-table experiments with *Daphnia galatea* fed two species of green algae showed that *Oocystis lacustris* was a lower quality food than *Scenedesmus acutus*, but only at relatively low food concentrations (Vanni & Lampert, 1992). The toxicity of chemicals often increases with food limitation, or nutritional deficiency, but sometimes the reverse is true. Barry *et al.* (1995) observed different effects of algal food concentration on the toxicity of agricultural pesticides to *D. magna*. The toxicity of the organochlorine pesticide endosulfan was greatest at higher food concentrations but for the synthetic pyrethroid esfenvalerate, toxicity increased significantly with decreasing food

concentrations. The literature data presented above provides strong evidence that the effect of environmental variables on the toxicity of chemicals is both species and chemical specific.

The potential value of the life-table approach

The value of life tables to study the potential long-term effects of exposure to chemicals has been demonstrated with several species of invertebrates including marine copepods (Daniels & Allan, 1981; Allan & Daniels, 1982; Bechmann, 1994), mysid shrimps (Gentile *et al.*, 1982, 1983), rotifers (Ferrando *et al.*, 1993; Janssen *et al.*, 1993, 1994) and cladocerans (Van Leeuwen *et al.*, 1985; Meyer *et al.*, 1987; Kluttgen *et al.*, 1996). The population parameter r_m has been proposed as a test parameter because it integrates age-specific survival and reproduction (age at first reproduction, reproductive frequency, brood size and reproductive period), thereby, providing a more ecologically relevant criterion than currently used toxicity test methods using separate measures of survival and reproduction. Current chronic test procedures focus on the total numbers of offspring produced and the significance of age at which reproduction first occurs is often underestimated (Van Leeuwen *et al.*, 1985). Age at first reproduction and offspring production both contribute to r_m but delays in the onset of reproduction can be more critical than the number of offspring produced (Allan, 1976; Daniels & Allan, 1981). Significant increases in day of first reproduction without concomitant decreases in brood size and longevity were reported for the copepod *Eurytemora affinis* exposed to dieldrin (Daniels & Allan, 1981) and for the mysid shrimp *Americamysis bahia* exposed to mercury and nickel (Gentile *et al.*, 1982). A possible objection to the life-table approach is the length of time required to complete life-table analysis, however, the length of exposure period is dependent upon temperature and test organism. For example, life-table analysis for *Tisbe battagliai* at temperatures of 15, 20 and 25°C required approximately 120, 94 and 39 days respectively (Chapter 4). Alternatively, life-table experiments with the

rotifer *Brachionus calyciflorus* required approximately 10 days at 25°C (Ferrando *et al.*, 1993). Due to the overiding importance of early reproduction, in terms of population growth and competition (Daniels & Allan, 1981), several authors have examined whether accurate estimates of r_m could be obtained from shorter exposure durations instead of waiting until all the animals have died. Life-table evaluation of the response of *Eurytemora affinis* to pesticides required exposure durations up to 60 days, however, accurate estimates of r_m were obtained from measurements taken after 21 days at 20°C (Allan & Daniels, 1981) or 30 days at 18°C (Daniels & Allan, 1982). For the cladoceran *Daphnia pulex*, data collected after 21 days provided accurate estimates of r_m after 56 days exposure to dieldrin (Daniels & Allan, 1982) and 70 days exposure to copper and cadmium (Meyer *et al.*, 1987). Meyer *et al.* (1987) emphasised the importance of early survival and reproduction in exponentially growing populations but noted that differences in survival and reproduction that occur later in life will be more important in stable ($r_m = 0$) or declining ($r_m < 0$) populations and in some fluctuating environments.

Unlike the complete life-cycle exposures described in Chapters 4 and 5 (which follows the fate of all individuals), the modified life table used in the experiments described in this chapter did not take into account juvenile survival (which impacts on both male and females) and the fate of those females that do not become reproductive. These two aspects will undoubtedly influence population parameters, therefore, the results from the modified life table should be treated with caution as they can potentially underestimate effects. This is particularly relevant to those treatments which received a unialgal diet of 1300 $\mu gC\ l^{-1}$ as present results have clearly showed relatively high levels of juvenile mortality and female infertility.

Bechmann (1994) reported reductions in r_m for *Tisbe furcata* at copper concentrations representing 32% of the 96 h LC50 value and, comparing these results with published data,

noted the relatively small difference between concentrations causing mortality in acute tests and those reducing r_m in life-table experiments. One possible explanation (Bechmann, 1994) was that organisms are able to detoxify substances when exposed to low concentrations, and, therefore, no effects are seen at the population level even though the substance may be bioavailable. PCP had a significant effect on the demographic parameters of *T. battagliai* at concentrations representing approximately 10% of the 96 h LC50 value on the low ration (1300 µgC l^{-1}) algal diet but no effects were observed at concentrations representing 30% of the 96 h LC50 for copepods fed the high ration (3250 µgC l^{-1}) algal diet. The results for *T. battagliai* and those for *T. furcata* (Bechmann, 1994) suggest that r_m is affected significantly at acute, but not at sublethal, exposure concentrations. There are, however, a number of important factors to consider before this assumption is validated. It is apparent from the *Tisbe* life-table data that relatively large reductions in certain demographic parameters are required to significantly reduce r_m. For example compared with the control, copepods exposed to 100 µg l^{-1} PCP and fed a low ration (1300 µgC l^{-1}) unialgal or mixed species algal diet showed large and significant reductions for lifespan (39 - 42%), reproductive period (61 - 62%), number of broods (48 - 50%) and numbers of offspring (47 - 66%). These reductions in demographic parameters contributed to a reduction (19.5 - 23.7%) in r_m compared with the control, however, the net reproductive rate (R_o) indicated an approximate twenty fold increase in population size from one generation to another. By comparison, life-table data for *T. furcata* (Bechmann, 1994) revealed a large reduction (66%) in r_m compared with the control, however, r_m was still positive (population was increasing) and R_o indicated a five fold increase in population size from one generation to another (compared with R_o = 29 for control). These data showed that the copepod populations were still able to increase, despite relatively large reductions in r_m. This does not imply that life-table analysis is insensitive but results will require careful interpretation. For environmental hazard assessment, negative values of r_m,

(indicating that populations will become extinct) unquestionably indicate potentially severe effects. However, what judgements can be made concerning the magnitude of reductions in r_m noted above? Measurements of r_m are usually based on data from one cohort of organisms per toxicant exposure concentration and do not report standard errors or confidence intervals on values of r_m, therefore, precluding statistical inferences about differences in r_m between treatments and the control. Statistical methods for measuring uncertainty in r_m from individual cohorts of cladocerans have been described (Meyer *et al.*, 1986, 1987) and, although such methods are computer-intensive, they are needed to provide statistical comparisons between the data.

In the experiments described in this chapter, measurements of reproduction (e.g. offspring production) for individually-held *Tisbe battagliai* showed wide variation and variation was most pronounced at the lower food ration. These findings are in agreement with previous experiments (Chapters 3 and 4) and indicate the inherent biological variability in *T. battagliai*. High levels of individual variability in survival and reproductive parameters have been reported also by Bechmann (1994) for *Tisbe furcata*, by Martinez-Jeronimo *et al.* (1994) for *Daphnia magna* and by Koivisto and Ketola (1995) for *Bosmina longirostris*. The selection of test organisms with limited genetic heterogeneity (e.g. *Daphnia* sp.) has been criticised as a means of reducing the inherent biological variability of the test system (Forbes & Depledge, 1992). Genetic homogeneity in test organisms may reduce individual variability and, thereby, improve the statistical power of the test for detecting significant differences compared with the control. However, such tests may have very limited ecological significance as biological variability is an important component of the population's ecological and evolutionary response to pollution stress (Forbes & Forbes, 1994). In these *Tisbe* experiments, high levels of variability in the response of copepods fed a low food ration may have masked the effects of PCP. For example, there was a large reduction (47 - 63%) in

offspring production compared with the control, for copepods exposed to 32 and 100 μg l^{-1} PCP and fed 1300 μgC l^{-1} of *Isochrysis galbana* (Table 6.13). These differences were not statistically significant but reduced fecundity contributed to a 24 - 31% reduction in r_m. In these circumstances, life-table approaches can provide valuable information on the population response to toxicants even if individual variation in survival and reproduction do not enable detection of statistically significant differences between treatments and the control.

Table 6.1. The acute and chronic toxicity of PCP to *Tisbe battagliai* at different temperature and food regimes. Experimental design for preliminary and definitive experiments.

Exposure regime	Parameter	Test† status	Temp (°C)	Algal conc. ($\mu gC\ l^{-1}$)	Test concentration ($\mu g\ l^{-1}$)
Acute	Survival	D	15, 20, 25	0, 3250	Control, Solvent control, 180, 320, 560, 1000, 1800
Chronic	Development	P	15	520, 1300, 3250	Control, Solvent control, 18, 32, 56, 100, 180, 320
			20	520, 1300, 3250	Control, Solvent control, 56, 100, 180, 320, 560, 1000
Chronic	Development	D	15, 20, 25	1300, 2055, 3250	Solvent control, 10, 32, 100
Chronic	Reproduction	P	15	520, 1300, 3250	Control, Solvent control, 18, 32, 56, 100, 180, 320, 560
			20	520, 1300, 3250	Control, Solvent control, 56, 100, 180, 320, 560, 1000
Chronic	Reproduction	D	15, 20, 25	1300, 2055, 3250	Solvent conrol, 10, 32, 100
Chronic	Life cycle	D	20	1300, 3250 *	Solvent conrol, 10, 32, 100

† D denotes definitive experiment, P denotes preliminary experiment.

* In complete life-cycle exposures, experiments were performed with unialgal (*Isochrysis galbana*) and mixed algal (*I. galbana* and *Rhodomonas reticulata*) diets. All other experiments were performed with a unialgal diet (*Isochrysis galbana*).

Table 6.2. The effect of temperature (15, 20 & 25°C) on the acute toxicity of PCP to the nauplius and adult female life stages of *Tisbe battagliai*. Comparison of results is made between fed (3250 µgC l^{-1} of algae) and unfed (0 µgC l^{-1}) treatments. LC50 values were calculated using the moving average angle or the binomial method (n.d., values could not be determined).

Temp. (°C)	Life stage	Time (h)	LC50 in µg l^{-1} (95% CI)			
			Unfed		Fed	
15	Nauplius	24	859	(743-1009)	753	(649-875)
		48	753	(649-875)	753	(649-875)
		72	632	(531-762)	709	(596-866)
		96	534	(446-636)	617	(519-742)
	Adult female	24	748	(560-1000)	748	(560-1000)
		48	709	(596-866)	748	(560-1000)
		72	534	(446-636)	556	(466-664)
		96	478	(395-567)	499	415-593)
20	Nauplius	24	1116	(995-1266)	1090	(972-1234)
		48	791	(682-922)	870	(752-1024)
		72	514	(428-611)	735	(617-903)
		96	394	(338-456)	592	(497-709)
	Adult female	24	647	(544-781)	684	(575-832)
		48	397	(340-460)	486	(403-577)
		72	300	(266-335)	361	(307-418)
		96	294	(260-328)	338	(284-391)
25	Nauplius	24	>1800	-	>1800	n.d.
		48	801	(670-969)	>1800	n.d.
		72	579	(474-709)	>1800	n.d.
		96	466	(385-554)	1023	(910-1152)
	Adult female	24	1044	(930-1178)	1116	(995-1266)
		48	722	(606-884)	1067	(951-1205)
		72	558	(467-666)	731	(614-897)
		96	403	(346-467)	541	(453-645)

Table 6.3. Three-way analysis of variance (ANOVA) of the effect of temperature (15, 20 and 25°C), food (algal concentrations of 1300, 2055 and 3250 µgC l^{-1}) and PCP (0, 10, 32 and 100 µg l^{-1}) on offspring production and the adult development time (days) of *Tisbe battagliai* (n.s., not significant; * significant at the 5% level; ** significant at the 1% level).

Source of variation	d.f.	Sum of squares	Mean square	F-ratio	Significance level
OFFSPRING PRODUCTION					
Temperature	2	14948.3	7474.1	12.9	**
Algae	2	108477.0	54238.7	93.5	**
PCP	3	6311.3	2103.8	3.63	*
T x A	4	12428.4	3107.1	5.36	**
A x PCP	6	4572.7	762.1	1.31	n.s.
T x PCP	6	7541.9	1257	2.17	*
Error	336	194928	580.1		
Total	359	349208			
HATCHING TO ADULT (N1-A)					
Temperature	2	2506	1253	280.3	**
Algae	2	13.3	6.63	1.48	n.s.
PCP	3	58.1	19.4	4.34	**
T x A	4	34.8	8.70	1.95	n.s.
A x PCP	6	4.2	0.69	0.15	n.s.
T x PCP	6	123.5	20.6	4.61	**
Error	336	1502.1	4.47		
Total	359	4241.9			

Table 6.4. Stage durations (days) for the development from hatching to adult (N1-A) for *Tisbe battagliai* reared at 15°C, algal food concentrations of 1300, 2055 and 3250 µgC l^{-1} and exposed to PCP concentrations of 0 (solvent control), 10, 32 and 100 µg l^{-1}. Data represent the mean values for surviving copepods (out of 10) that successfully developed to the adult stage. Statistical analysis of the data (both sexes combined) revealed no differences in development times between the control and PCP treatments.

Sex	1300 µgC l^{-1}				2055 µgC l^{-1}				3250 µgC l^{-1}			
of copepod	µg PCP l^{-1}	n	Mean	(SD)	µg PCP l^{-1}	n	Mean	(SD)	µg PCP l^{-1}	n	Mean	(SD)
Male	0	7	14.3	(0.95)	0	6	14.3	(0.52)	0	3	14.3	(0.58)
	10	7	13.3	(0.76)	10	7	13.6	(0.53)	10	5	14.0	(0)
	32	8	13.1	(0.35)	32	3	13.7	(0.58)	32	4	14.0	(0)
	100	2	13.5	(0.71)	100	6	14.3	(0.52)	100	5	14.0	(0)
Female	0	3	16.3	(1.15)	0	4	15.3	(0.50)	0	7	15.0	(0.58)
	10	3	15.0	(1.00)	10	3	14.3	(0.58)	10	5	14.6	(0.89)
	32	2	14.5	(0.71)	32	7	14.3	(0.49)	32	6	14.0	(0)
	100	6	14.8	(1.17)	100	2	14.0	(0)	100	3	15.0	(0)
Both	0	10	14.9	(1.37)	0	10	14.7	(0.67)	0	10	14.8	(0.63)
	10	10	13.8	(1.14)	10	10	13.8	(0.63)	10	10	14.3	(0.67)
	32	10	13.4	(0.70)	32	10	14.1	(0.57)	32	10	14.0	(0)
	100	8	14.5	(1.20)	100	8	14.3	(0.46)	100	8	14.4	(0.52)

Table 6.5. Stage durations (days) for the development from hatching to adult (N1-A) for *Tisbe battagliai* reared at 20°C, algal food concentrations of 1300, 2055 and 3250 µgC l^{-1} and exposed to PCP concentrations of 0 (solvent control), 10, 32 and 100 µg l^{-1}. Data represent the mean values for surviving copepods (out of 10) that successfully developed to the adult stage (* significant at the 5% level compared with the control).

Sex	1300 µgC l^{-1}				2055 µgC l^{-1}				3250 µgC l^{-1}			
of copepod	µg PCP l^{-1}	n	Mean	(SD)	µg PCP l^{-1}	n	Mean	(SD)	µg PCP l^{-1}	n	Mean	(SD)
Male	0	4	9.5	(0.58)	0	6	9.83	(0.41)	0	2	9.00	(0)
	10	5	10.0	(0)	10	5	9.20	(0.84)	10	3	9.00	(0)
	32	1	11.0	(0)	32	8	9.00	(0)	32	3	9.33	(0.58)
	100	4	11.0	(0.81)	100	2	10.5	(2.12)	100	3	9.00	(0)
Female	0	6	11.3	(0.82)	0	4	10.0	(0)	0	8	9.75	(0.46)
	10	5	12.0	(0)	10	5	9.80	(0.45)	10	7	9.29	(0.49)
	32	8	12.1	(0.35)	32	2	10.0	(0)	32	7	9.57	(0.53)
	100	5	12.6	(0.89)	100	8	10.6	(0.52)	100	6	10.2	(0.41)
Both	0	10	10.6	(1.17)	0	10	9.90	(0.32)	0	10	9.60	(0.52)
	10	10	11.0	(1.05)	10	10	9.50	(0.71)	10	10	9.20	(0.42)
	32	9	12.0 *	(0.50)	32	10	9.20	(0.42)	32	10	9.50	(0.53)
	100	9	11.9 *	(1.17)	100	10	10.6	(0.84)	100	9	9.78	(0.67)

Table 6.6. Stage durations (days) for the development from hatching to adult (N1-A) for *Tisbe battagliai* reared at 25°C, algal food concentrations of 1300, 2055 and 3250 µgC l^{-1} and exposed to PCP concentrations of 0 (solvent control), 10, 32 and 100 µg l^{-1}. Data represent the mean values for surviving copepods (out of 10) that successfully developed to the adult stage. Statistical analysis of the data (both sexes combined) revealed no differences in development times between the control and PCP treatments.

Sex	1300 µgC l^{-1}				2055 µgC l^{-1}				3250 µgC l^{-1}			
of copepod	µg PCP l^{-1}	n	Mean	(SD)	µg PCP l^{-1}	n	Mean	(SD)	µg PCP l^{-1}	n	Mean	(SD)
Male	0	4	7.00	(0)	0	6	7.00	(0.63)	0	6	6.83	(0.41)
	10	8	7.00	(0.53)	10	6	7.17	(0.41)	10	7	6.71	(0.76)
	32	7	6.71	(0.49)	32	5	7.00	(0)	32	6	7.17	(0.75)
	100	4	7.00	(0)	100	5	7.00	(0)	100	6	7.00	(0.63)
Female	0	6	7.33	(0.52)	0	4	7.25	(0.50)	0	4	7.50	(0.58)
	10	2	7.50	(0.71)	10	4	7.25	(0.50)	10	3	7.00	(0)
	32	3	7.67	(0.58)	32	5	7.40	(0.55)	32	4	6.75	(0.50)
	100	6	7.33	(0.52)	100	5	7.20	(0.45)	100	4	7.00	(0)
Both	0	10	7.20	(0.42)	0	10	7.10	(0.57)	0	10	7.10	(0.57)
	10	10	7.00	(0.47)	10	10	7.20	(0.42)	10	10	6.80	(0.63)
	32	10	7.10	(0.57)	32	10	7.20	(0.42)	32	10	7.00	(0.67)
	100	10	7.20	(0.42)	100	10	7.10	(0.32)	100	10	7.00	(0.47)

Table 6.7. Adult size (body length in μm) of *Tisbe battagliai* reared at 15, 20 and 25°C, algal concentrations of 1300, 2055 and 3250 μgC l^{-1} and exposed to PCP concentrations of 0, 10, 32 and 100 μg l^{-1}. Results are expressed as the mean ±1SD and the values in parentheses denote the number of copepods (n). The data were not statistically analysed due to the small numbers of copepods (Section 6.3.3.1.2).

PCP conc. (μg l^{-1})	Temp. (°C)	Sex	Algal concentration (μgC l^{-1})		
			1300	2055	3250
0	15	Male	557 ±18 (6)	547 ±19 (6)	580 ±27 (3)
		Female	670 ±57 (4)	725 ±34 (4)	750 ±19 (7)
	20	Male	540 ±14 (4)	548 ±12 (6)	540 ±28 (2)
		Female	628 ±25 (6)	668 ±22 (4)	681 ±22 (8)
	25	Male	538 ±28 (4)	548 ±08 (6)	562 ±19 (6)
		Female	668 ±46 (6)	685 ±17 (4)	693 ±43 (4)
10	15	Male	538 ±13 (6)	544 ±22 (7)	564 ±09 (5)
		Female	660 ±36 (4)	713 ±21 (3)	726 ±30 (5)
	20	Male	506 ±15 (5)	526 ±24 (5)	543 ±21 (3)
		Female	638 ±05 (5)	618 ±28 (5)	676 ±30 (7)
	25	Male	521 ±31 (8)	523 ±26 (6)	540 ±31 (7)
		Female	625 ±07 (2)	685 ±34 (4)	713 ±15 (3)
32	15	Male	539 ±14 (8)	550 ±10 (3)	560 ±22 (4)
		Female	680 ±14 (2)	723 ±18 (7)	747 ±21 (6)
	20	Male	530 ±00 (1)	541 ±15 (8)	535 ±06 (4)
		Female	634 ±28 (8)	640 ±28 (2)	675 ±25 (6)
	25	Male	520 ±19 (7)	536 ±18 (5)	543 ±10 (6)
		Female	610 ±10 (3)	668 ±28 (5)	708 ±10 (4)
100	15	Male	560 ±14 (2)	547 ±18 (6)	560 ±20 (5)
		Female	680 ±35 (6)	720 ±14 (2)	737 ±31 (3)
	20	Male	503 ±17 (4)	543 ±15 (3)	513 ±21 (3)
		Female	636 ±23 (5)	659 ±24 (7)	655 ±33 (6)
	25	Male	525 ±06 (4)	520 ±27 (5)	533 ±15 (6)
		Female	642 ±15 (6)	672 ±16 (5)	688 ±19 (4)

Table 6.8. Complete life-cycle experiments with *Tisbe battagliai*; the number and sex ratio of copepods in each cohort (n=50) that successfully reached sexual maturity [* denotes a significant ($P < 0.05$) departure in sex ratio from 1:1].

Algal conc. ($\mu gC\ l^{-1}$)	Diet	PCP ($\mu g\ l^{-1}$)	% Dead	Sex ratio (no.) Male	Female	% Male
3250	*Isochrysis*	0	0	27	23	54
		10	8	21	25	46
		32	0	25	25	50
		100	8	25	21	54
3250	*Isochrysis &*	0	0	24	26	48
	Rhodomonas	10	0	23	27	46
		32	0	26	24	52
		100	0	24	26	48
1300	*Isochrysis*	0	12	28	16	64
		10	20	21	19	53
		32	6	33	14	70*
		100	24	23	15	61
1300	*Isochrysis &*	0	0	31	19	62
	Rhodomonas	10	0	28	22	56
		32	0	22	28	44
		100	0	25	25	50

Table 6.9. Complete life-cycle experiments with *Tisbe battagliai*; the number of fertile females in each cohort and the time taken for each female to produce their first egg sac [† significant ($P < 0.05$) increase in development time compared with corresponding value for mixed algal diet; * significant ($P < 0.05$) increase in development time compared with corresponding value at 3250 μgC l^{-1}].

Algal conc. (μgC l^{-1})	Diet	PCP (μg l^{-1})	Survival to adult (%)	Females		Egg-producing females		Time (d) to first egg sac
				%	(n)	%	(n)	mean (±SD)
3250	*Isochrysis*	0	100	46	(23)	96	(22)	12.5 (0.67)†
		10	92	54	(25)	100	(25)	13.2 (1.41)†
		32	100	50	(25)	96	(24)	13.3 (1.43)†
		100	92	46	(21)	95	(20)	12.6 (0.60)†
3250	*Isochrysis* &	0	100	52	(26)	100	(26)	11.2 (0.65)
	Rhodomonas	10	100	54	(27)	100	(27)	11.4 (0.70)
		32	100	48	(24)	100	(24)	11.1 (0.72)
		100	100	52	(26)	100	(26)	11.3 (0.92)
1300	*Isochrysis*	0	88	36	(16)	56	(9)	18.7 (3.97)†*
		10	80	47	(19)	63	(12)	18.4 (1.73)†*
		32	94	30	(14)	64	(9)	21.3 (3.00)†*
		100	76	39	(15)	67	(10)	18.3 (1.34)†*
1300	*Isochrysis* &	0	100	38	(19)	89	(17)	13.6 (2.12)*
	Rhodomonas	10	100	44	(22)	91	(20)	15.1 (3.14)*
		32	100	56	(28)	96	(27)	15.0 (2.62)*
		100	100	50	(25)	92	(23)	14.7 (2.34)*

Table 6.10. Summary of results from cohort life-table experiments with female *Tisbe battagliai* (n = 10). Females were exposed to different concentrations of PCP at 20°C and fed 3250 µgC l^{-1} of a mixed algal diet consisting of *Isochrysis galbana* and *Rhodomonas reticulata*. Data represent the mean ±1SD except for T_c, R_o, and r_m which were calculated from the survival and fecundity schedules (* significant at the 5% level compared with the control).

Parameter	PCP concentration (µg l^{-1})			
	0	10	32	100
Lifespan (days)	56.5 ±10.2	51.8 ±15.7	51.9 ±17.6	51.1 ±12.3
Reproductive period (days)	23.4 ±5.15	17.0 ±6.50*	21.5 ±5.95	17.2 ±7.00*
Total no. of broods	8.90 ±1.52	6.30 ±2.00*	7.70 ±1.89	6.20 ±2.44*
Total no. of offspring	283 ±52.9	199 ±64.0*	250 ±80.8	188 ±65.7*
Offspring per female per reproductive day	12.4 ±2.64	12.6 ±3.14	12.0 ±3.78	11.4 ±2.34
T_{min} (days)	13.2 ±0.42	13.6 ±0.97	13.0 ±0	13.1 ±0.32
T_c (days)	23.0	21.2	22.2	21.2
R_o	141	99.4	125	94.9
r_m (d^{-1})	0.270	0.257	0.268	0.263

Table 6.11. Summary of results from cohort life-table experiments with female *Tisbe battagliai* (n = 10). Females were exposed to different concentrations of PCP at 20°C and fed 3250 µgC l^{-1} of a unialgal diet consisting of *Isochrysis galbana*. Data represent the mean ±1SD except for T_c, R_o, and r_m which were calculated from the survival and fecundity schedules (* significant at the 5% level compared with the control).

Parameter	PCP concentration (µg l^{-1})			
	0	10	32	100
Lifespan (days)	50.3 ±7.54	54.4 ±12.1	57.2 ±13.3	54.7 ±9.81
Reproductive period (days)	20.9 ±3.48	22.8 ±11.7	21.3 ±4.67	20.5 ±7.23
Total no. of broods	8.30 ±1.34	7.30 ±2.54	8.10 ±2.13	7.80 ±3.12
Total no. of offspring	251 ±58.8	192 ±80.7	260 ±73.6	202 ±80.0
Offspring per female per reproductive day	12.3 ±3.91	8.95 ±2.79*	12.2 ±2.12	10.0 ±2.33
T_{min} (days)	14.6 ±0.52	14.8 ±0.63	14.5 ±0.53	14.7 ±0.67
T_c (days)	24.4	25.8	24.7	26.7
R_o	127	95.9	137	100
r_m (d^{-1})	0.238	0.219	0.240	0.216

Table 6.12. Summary of results from cohort life-table experiments with female *Tisbe battagliai* (n = 10). Females were exposed to different concentrations of PCP at 20°C and fed 1300 µgC l^{-1} of a mixed algal diet consisting of *Isochrysis galbana* and *Rhodomonas reticulata*. Data represent the mean ±1SD except for T_c, R_o, and r_m which were calculated from the survival and fecundity schedules (* significant at the 5% level compared with the control).

Parameter	PCP concentration (µg l^{-1})			
	0	10	32	100
Lifespan (days)	59.0 ±19.2	54.4 ±25.0	43.3 ±4.60*	34.5 ±14.8*
Reproductive period (days)	26.5 ±6.98	23.4 ±12.4	16.2 ±9.10*	10.2 ±8.70*
Total no. of broods	6.70 ±2.16	6.60 ±2.80	4.20 ±2.20*	3.30 ±2.11*
Total no. of offspring	121 ±58.5	110 ±55.8	72.0 ±35.4	41.2 ±41.7*
Offspring per female per reproductive day	4.45 ±1.43	5.74 ±3.44	4.94 ±4.39	4.09 ±2.27
T_{min} (days)	15.4 ±0.70	16.2 ±1.32	15.9 ±0.88	15.9 ±1.10
T_c (days)	26.5	26.8	23.0	22.3
R_o	61.1	57.7	36.7	20.6
r_m (d^{-1})	0.195	0.185	0.186	0.157

Table 6.13. Summary of results from cohort life-table experiments with female *Tisbe battagliai* (n = 10). Females were exposed to different concentrations of PCP at 20°C and fed 1300 µgC l^{-1} of a unialgal diet consisting of *Isochrysis galbana*. Data represent the mean ±1SD except for T_c, R_o, and r_m which were calculated from the survival and fecundity schedules (* significant at the 5% level compared with the control).

Parameter	PCP concentration (µg l^{-1})			
	0	10	32	100
Lifespan (days)	57.0 ±18.0	46.0 ±9.80	38.7 ±9.72*	34.7 ±10.1*
Reproductive period (days)	22.4 ±13.1	15.7 ±9.38	8.33 ±8.44*	8.80 ±9.54*
Total no. of broods	5.78 ±2.91	4.30 ±2.06	2.67 ±2.06*	2.70 ±2.45*
Total no. of offspring	76.9 ±61.8	61.3 ±43.9	28.8 ±28.0	40.5 ±48.6
Offspring per female per reproductive day	2.95 ±1.49	3.12 ±2.20	1.85 ±1.93	2.26 ±2.88
T_{min} (days)	21.1 ±4.00	20.5 ±2.17	23.6 ±3.38	21.9 ±3.83
T_c (days)	31.4	29.9	30.7	31.9
R_o	38.8	30.6	14.4	19.9
r_m (d^{-1})	0.131	0.126	0.091	0.10

Table 6.14. Two-way analysis of variance (ANOVA) of the effect of PCP (0, 10, 32 and 100 μg l^{-1}) and algal diet (unialgal and mixed species algal diets, each at 1300 and 3250 μgC l^{-1}) on the total number of offspring produced by *Tisbe battagliai* (n.s., not significant; * significant at the 5% level; ** significant at the 1% level).

Source of variation	d.f.	Sum of squares	Mean square	F-ratio	Significance level
OFFSPRING PRODUCTION					
PCP	3	81618	27206	7.87	**
Diet	3	1056200	352050	101.86	**
PCP x Diet	9	63925	7102.7	2.06	*
Error	135	466570	3456.1		
Total	159	1724600			

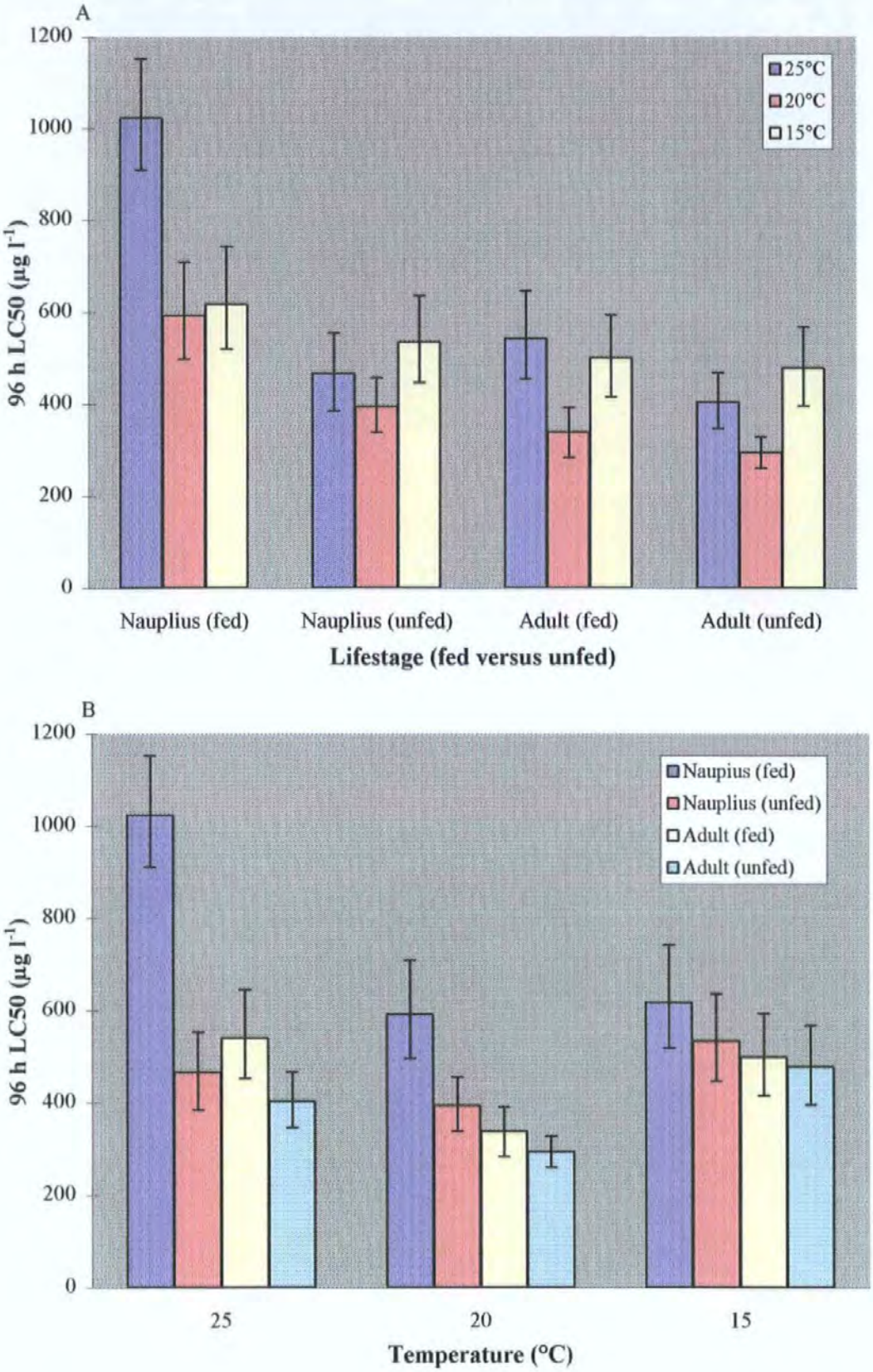
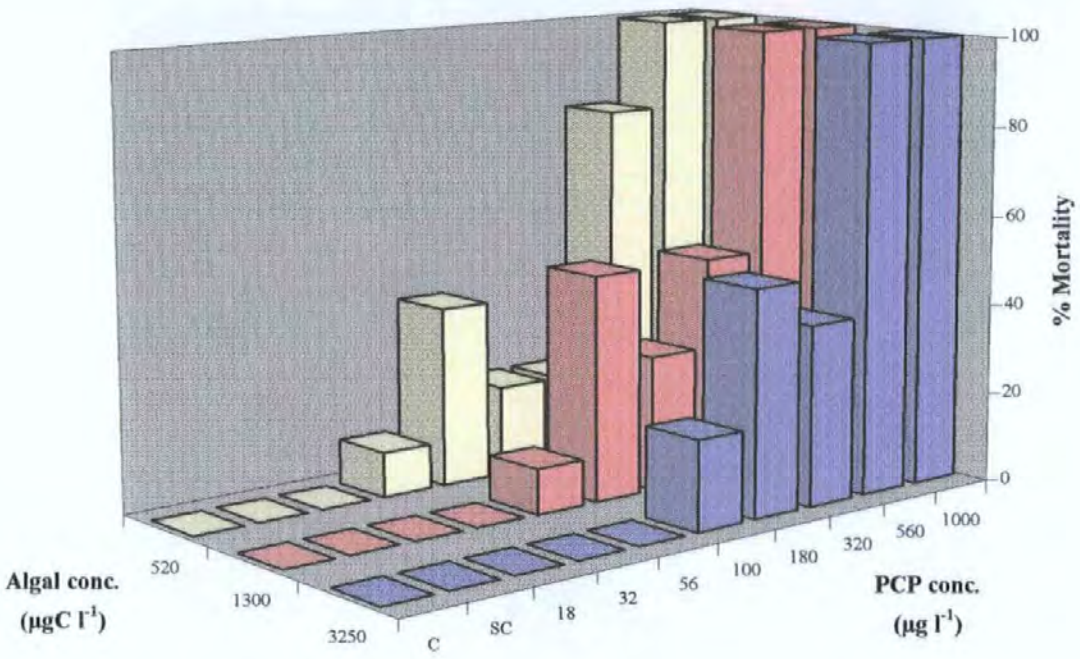


Figure 6.1. The acute toxicity of PCP to the nauplius and adult female life stages of *Tisbe battagliai* at 15, 20 and 25°C, with and without the addition of algae as food. Comparisons between the 96 h LC50 values (± 95% confidence intervals) are shown for (A) the effect of temperature and (B) the sensitivity of the different life stages.

A



B

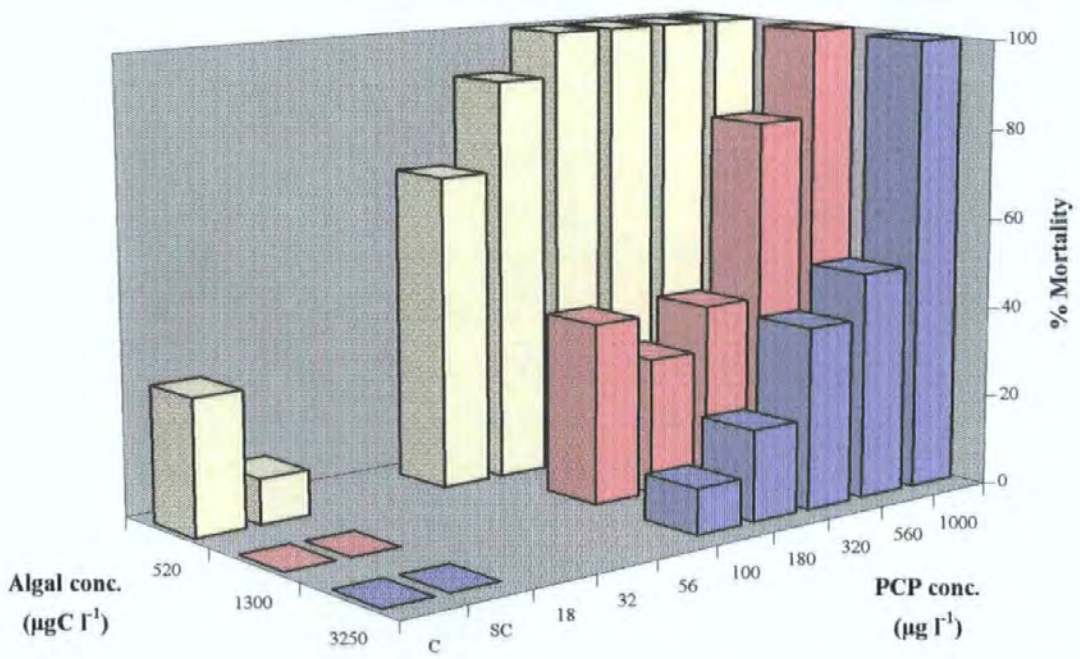


Figure 6.2. Mortality during development from hatching to the adult stage for *Tisbe battagliai* reared at different algal concentrations (520, 1300 and 3250 µgC l) and temperatures of (A) 15°C and (B) 20°C. Control (C) and solvent control (SC) treatments are identified as shown.

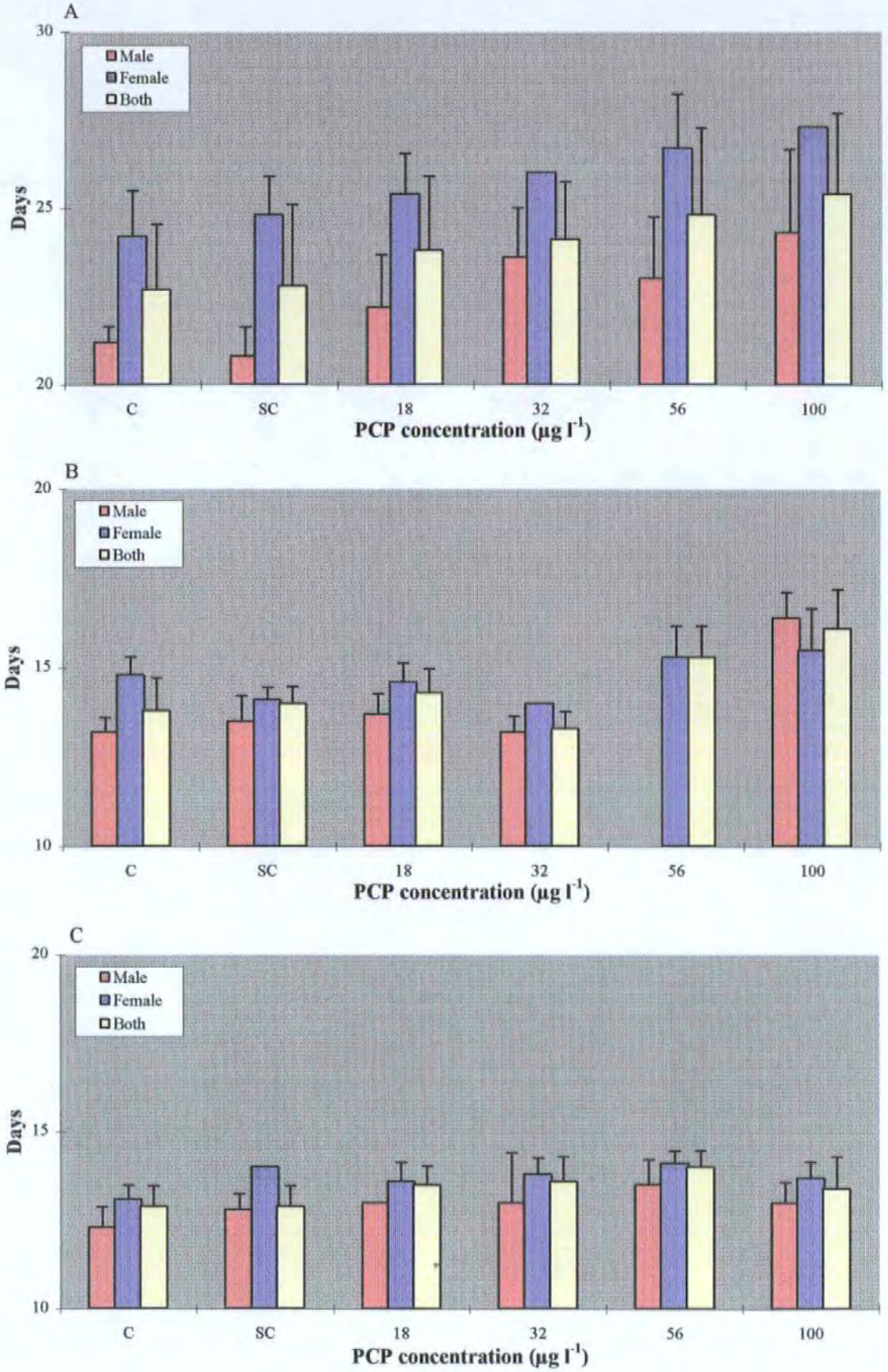


Figure 6.3. The effect of PCP on the postembryonic development of *Tisbe battagliai* reared at 15°C and algal concentrations of (A) 520, (B) 1300 and (C) 3250 µgC l. Values (mean ±1SD) represent the time taken (days) from hatching to reach the adult stage (N1-A).

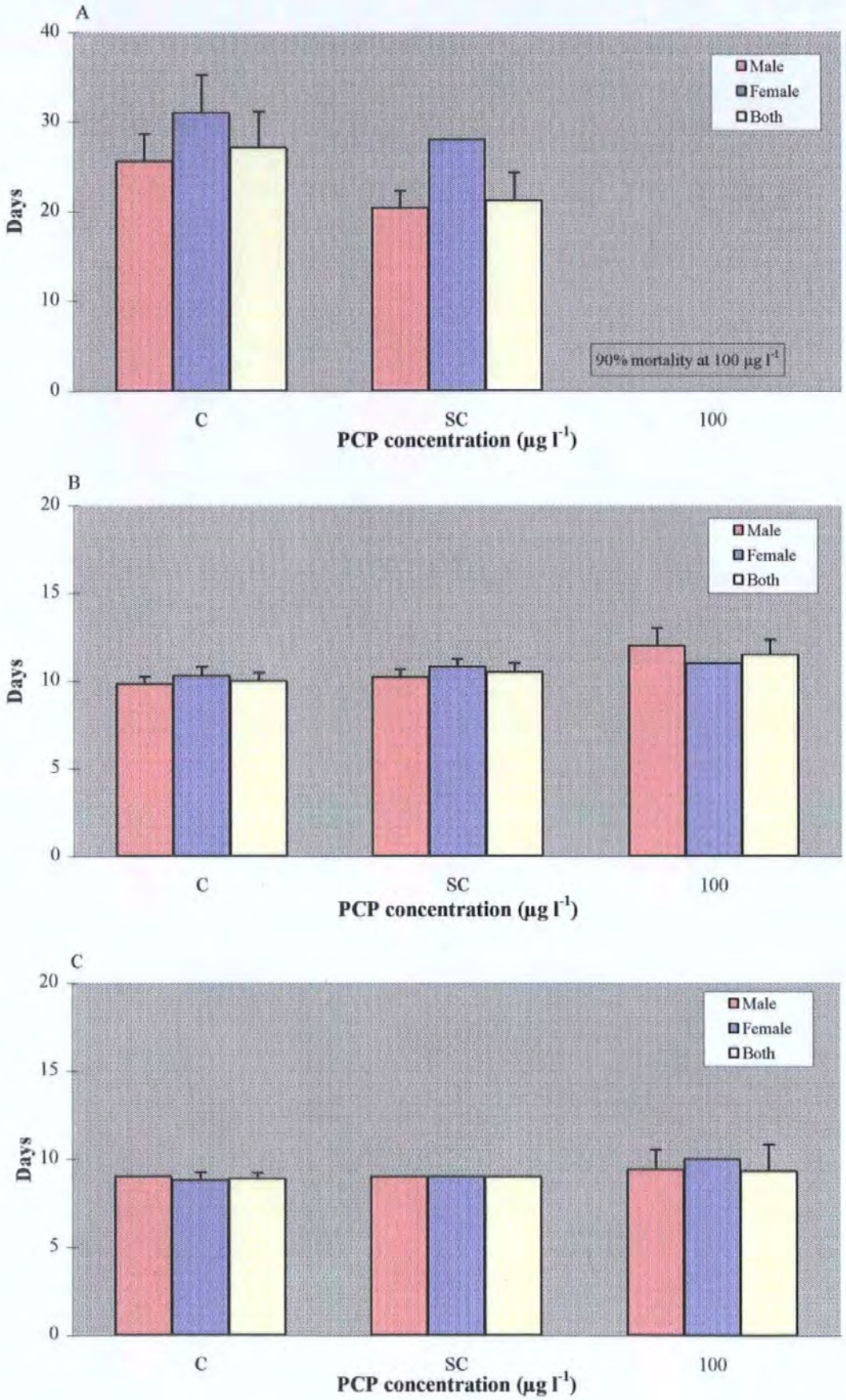


Figure 6.4. The effect of PCP on the postembryonic development of *Tisbe battagliai* reared at 20°C and algal concentrations of (A) 520, (B) 1300 and (C) 3250 µgC l. Values (mean ±1SD) represent the time taken (days) from hatching to reach the adult stage (N1-A).

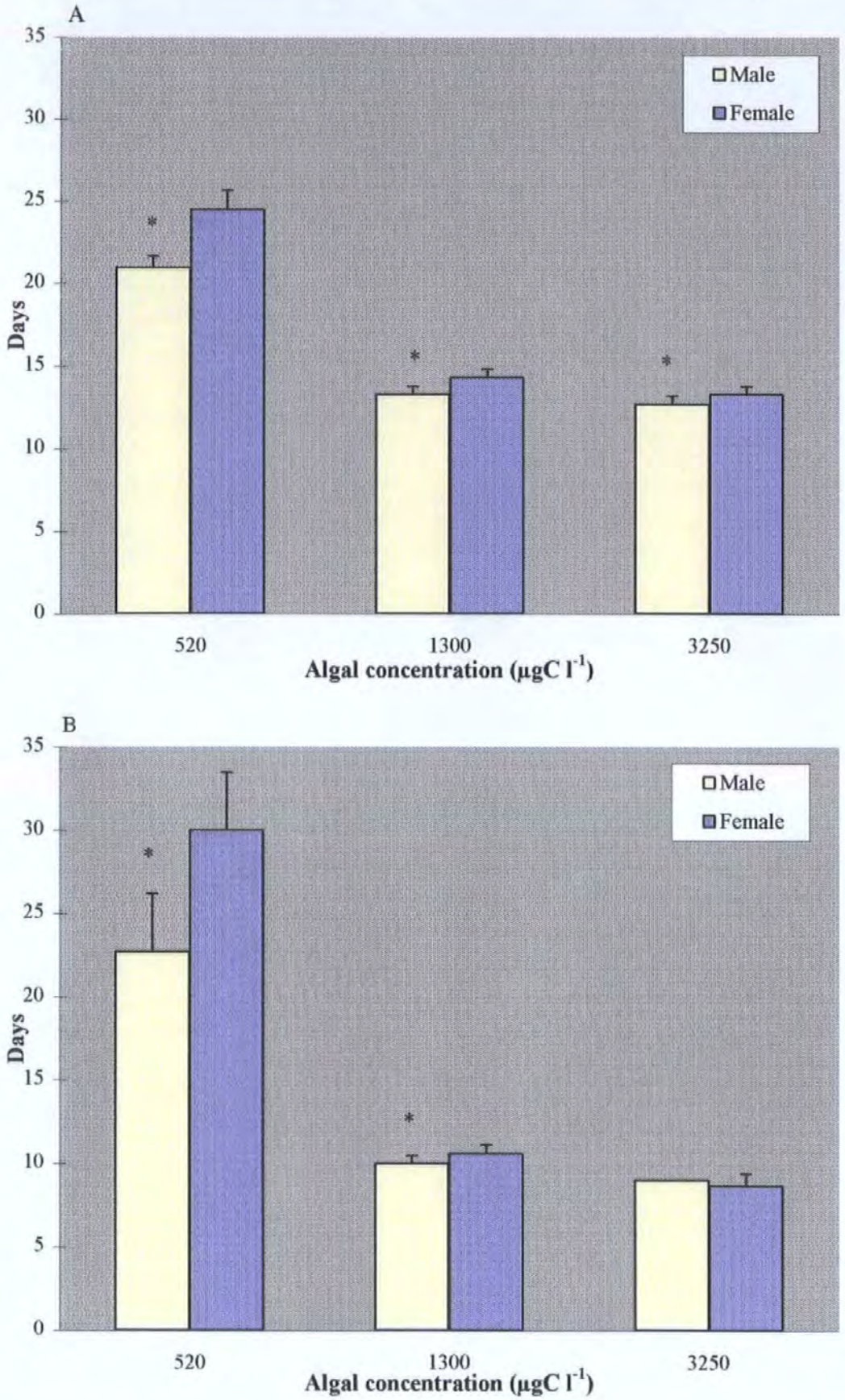


Figure 6.5. The time taken (days) for male and female *Tisbe battagliai* to reach the adult stage at algal concentrations of 520, 1300 and 3250 µgC l and temperatures of ((A) 15°C and (B) 20°C [values represent mean ± 1SD and * denotes a significant difference ($P < 0.05$) between the sexes].

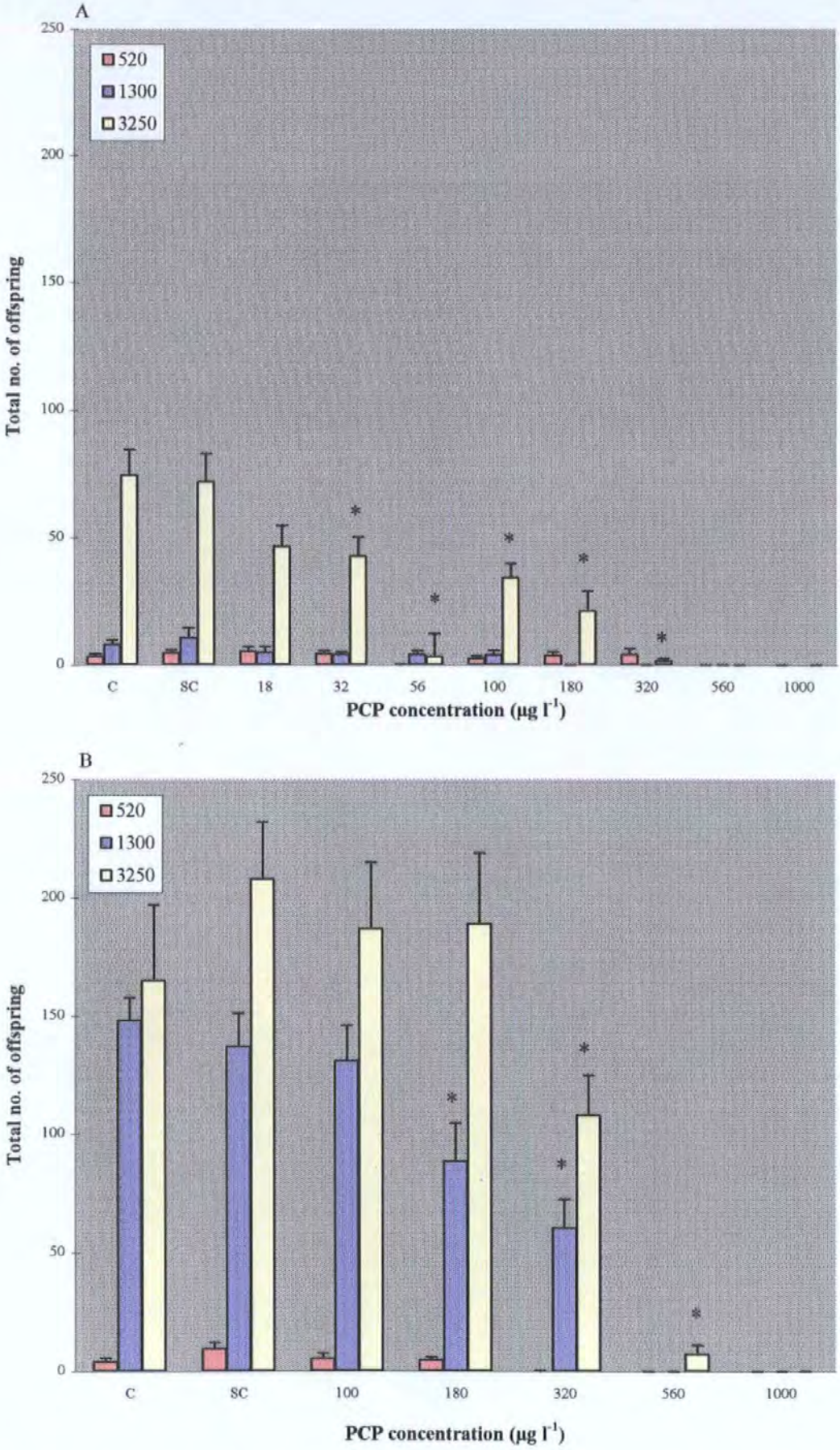


Figure 6.6. The effect of PCP on the reproduction of *Tisbe battagliai* reared at algal concentrations of 520, 1300 and 3250 µgC l and temperatures of (A) 15°C and (B) 20°C. Values represent mean ±1SE (n=10) and * denotes a significant difference ($P < 0.05$) compared with the solvent control (SC).

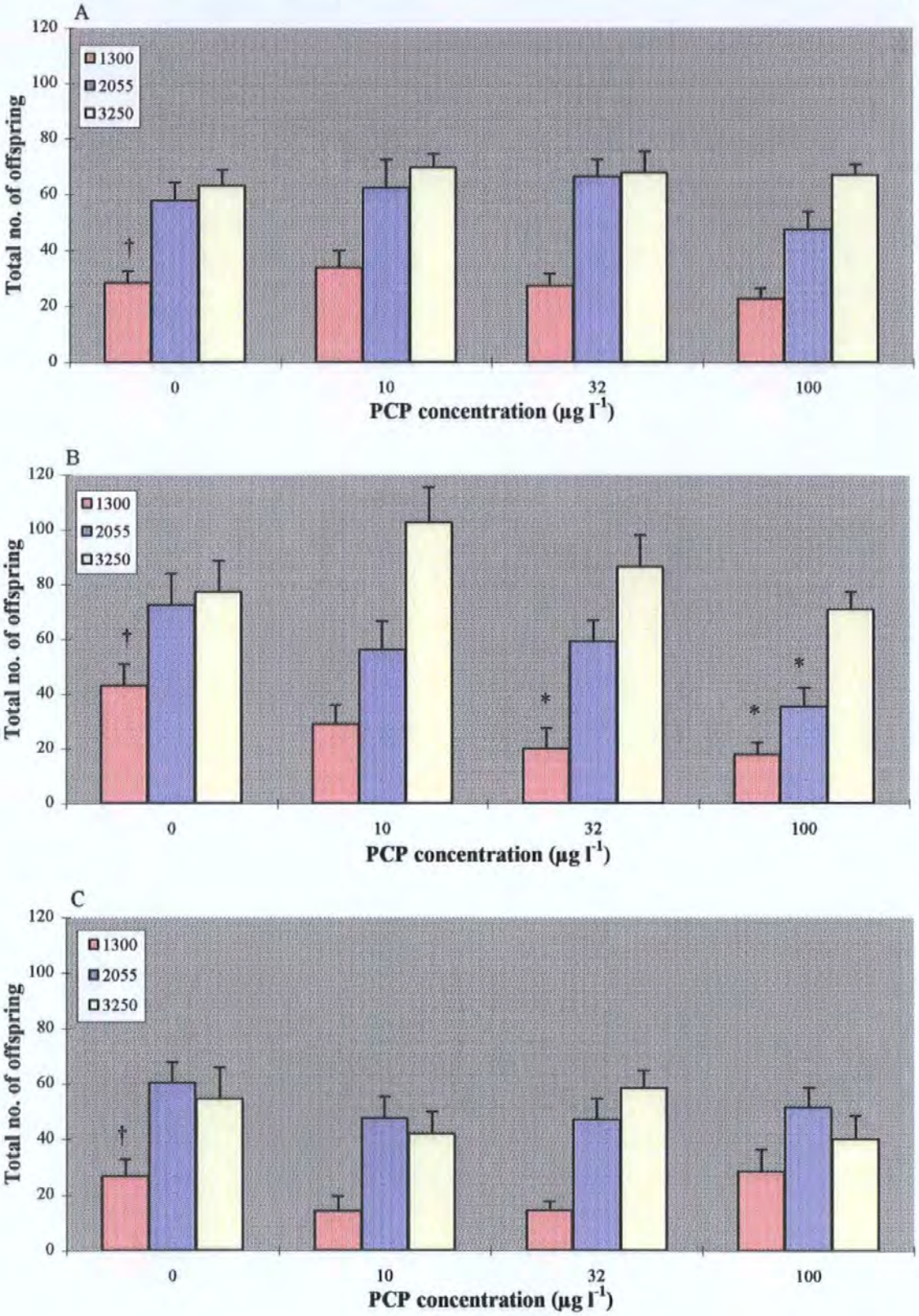


Figure 6.7. The effect of PCP on the reproduction of *Tisbe battagliai* at algal concentrations of 1300, 2055 and 3250 ugC l and temperatures of (A) 15°C, (B) 20°C and (C) 25°C. Values represent mean ±1SE (n=10) and * denotes a significant difference ($P < 0.05$) compared with the solvent control (0) and † denotes a significant ($P < 0.05$) reduction (control data only) compared with higher algal concentrations.

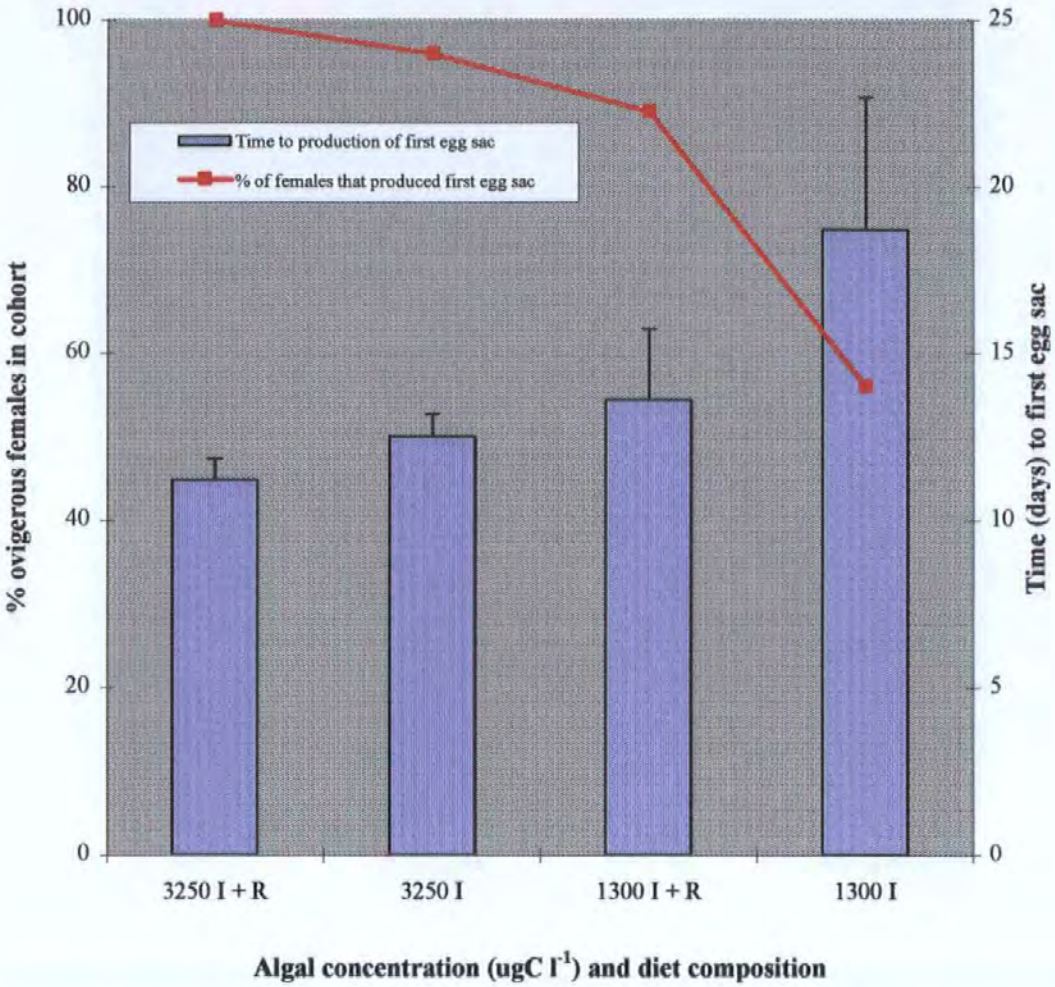


Figure 6.8. The number of ovigerous females and the time taken (days) to produce the first egg sac in cohorts of *Tisbe battagliai* fed algal diets consisting of 1300 and 3250 µgC l of either a unialgal or mixed species algal diet. Results represent mean values ±1SD (n=50).

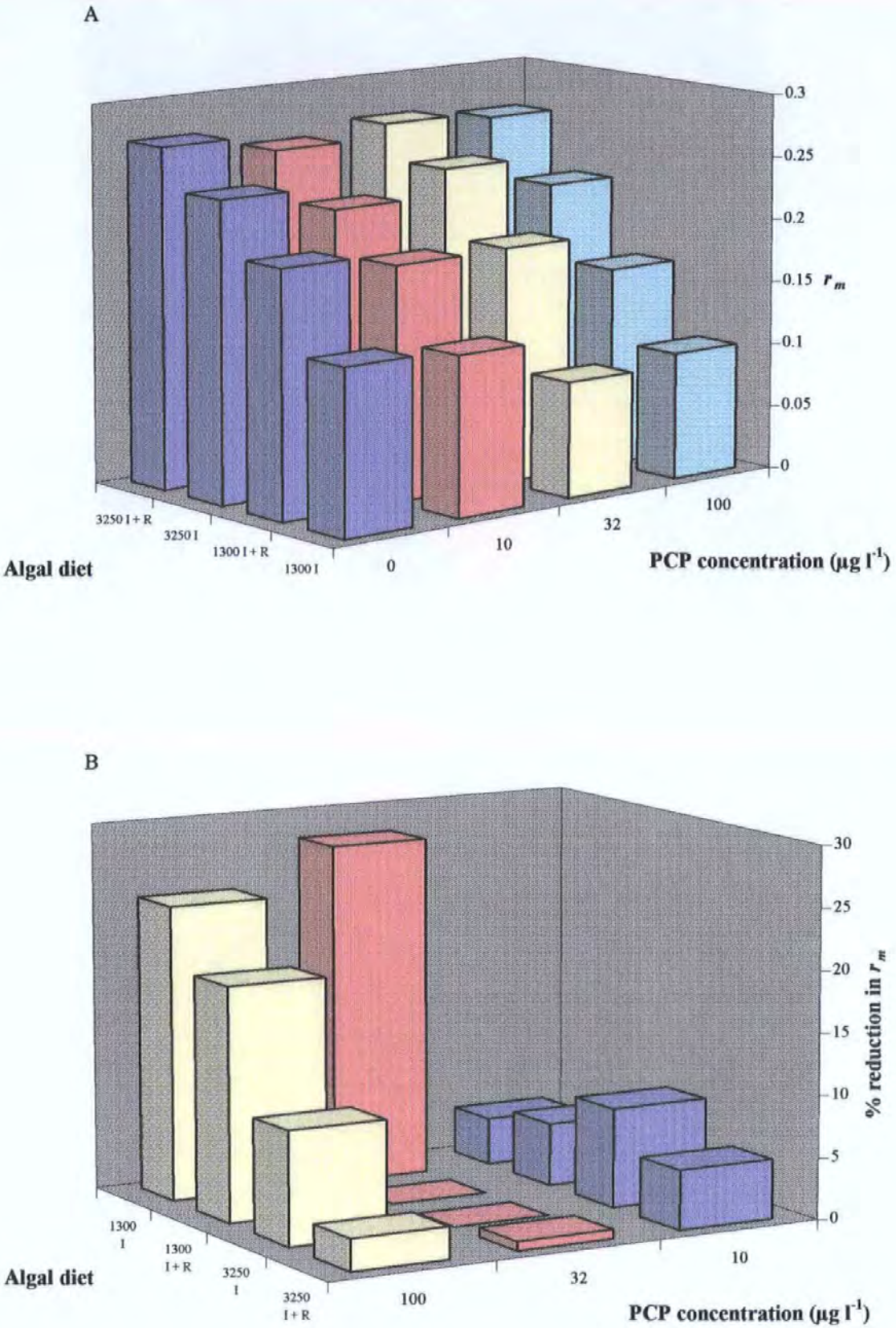


Figure 6.9. The effect of algal diet and PCP on the intrinsic rate of natural increase (r_m) for *Tisbe battagliai*. Results are expressed as (A) measured values of r_m and (B) the percentage reduction in r_m compared with the control. Algal diets consist of *Isochrysis* + *Rhodomonas* (I+R) or *Isochrysis* (I).

CHAPTER 7

THE EFFECT OF PENTACHLOROPHENOL ON THE POPULATION DYNAMICS OF *TISBE BATTAGLIAI*: MEASUREMENT OF EFFECTS ON POPULATION GROWTH

7.1. INTRODUCTION

A recurring theme in the ecotoxicological literature is the need for greater insight and understanding into the links between pollutant effects across different levels of biological organisation (Section 1.4.4). Established chronic toxicity test procedures are based on the measurement of individual organisms and tests are usually performed at high food levels. This approach to chronic toxicity testing does not take into account population effects that occur through interactions between individuals (competition and food availability) (Van Leeuwen *et al.*, 1985, 1987). There are relatively few studies reporting the effects of multigeneration exposure to chemicals, however, data suggest that the sublethal effects of chemicals may not be apparent within one generation. For example, Postma and Davids (1995) reported that the effects of cadmium on mortality, growth and reproduction of the chironomid *Chironomus riparius* increased during consecutive generations (even at concentrations which had no negative effect during the first generation). Towards the end of their experiment (exposure of nine consecutive generations), decreasing effects were observed on growth and reproduction, indicating an increased tolerance to cadmium (Postma & Davids, 1995). Verriopoulos and Hardouvelis (1988) reported a significant decrease in the production of egg sacs by four successive generations of *Tisbe holothuriae* exposed to sublethal concentrations of zinc. These studies (Verriopoulos & Hardouvelis, 1988; Postma & Davids, 1995) suggest that prolonged exposure to sublethal concentrations of chemicals may cause changes in population parameters that are not detected by experiments conducted over one generation. Consequently, in environmental hazard assessment, the derivation of 'safe concentrations' from toxicity experiments conducted over one generation may underestimate the potential hazard posed by exposure to chemicals over multiple generations of the same species. In population experiments, the exposure duration can be sufficiently long to encompass effects on more than

one generation of the test organism. Such investigations may provide a greater insight into the changes in population dynamics of species due to prolonged exposure to chemicals.

The aim of this chapter was to develop toxicity test procedures for measuring the potential long-term effect of chemicals on populations of *Tisbe battagliai* and to investigate whether population measurements respond differently from measurements taken from individual organisms. Following the approach of previous chapters, the influence of a key environmental factor (food quantity and quality) on the population response was also measured.

7.2. MATERIALS AND METHODS

The procedures for culturing *Tisbe battagliai*, and associated algal food species *Isochrysis galbana* and *Rhodomonas reticulata*, have been described previously (Chapters 2 and 5 respectively). Details of the test chemical (pentachlorophenol) and of the procedures used to prepare the test solutions are described in Chapter 6.

7.2.1. Test procedure

The experimental design for the population experiments was similar to that employed for complete life-cycle exposures (Chapter 6) with the following changes. Population experiments were carried out at a single temperature (20°C), and copepods were fed unialgal (*Isochrysis galbana*) and mixed species (*I. galbana* and *Rhodomonas reticulata*) algal diets, each at three different food concentrations (520, 1300 and 3250 $\mu gC\ l^{-1}$). Algal concentrations were selected to provide comparisons of population growth under conditions of limited (520 $\mu gC\ l^{-1}$) and unlimited (3250 $\mu gC\ l^{-1}$) food availability. Based on the results from previous

experiments (Chapter 6), and limitations in the size of the experimental design, tests were performed with a solvent control and a single concentration of PCP (100 μg l^{-1}). Previous experiments established that copepods exposed to conditions of food limitation showed an increased sensitivity to PCP at a concentration of 100 μg l^{-1} (Chapter 6). Population experiments were carried out in two stages. Firstly, development of the nauplii from hatching to production of the first egg sac was followed in the different treatments. Secondly, a proportion of the copepods that had developed to reproductive maturity in the different treatments was selected and used to initiate population experiments which continued for 77 days.

7.2.1.1. Development from hatching to production of the first egg sac

Cohorts of nauplii (< 24 h old) were exposed to different combinations of PCP (0 and 100 μg l^{-1}) and unialgal or mixed species algal diet until the adult stage was reached. Fifty nauplii were added randomly, in groups of ten each, to 5 wells of tissue culture plates, each containing 10 ml of test solution. Observations of mortality were made at 24 h intervals and the surviving copepods transferred to duplicate test chambers containing freshly prepared test solutions. The progress of nauplii was followed from hatching through to adulthood and the sex of each copepod, and the time taken for production of the first egg sac by females, was recorded. Temperature (daily), and the pH and dissolved oxygen concentration (at least weekly) was measured in each treatment.

7.2.1.2. Selection of adult stages to initiate population experiments

Following development to adulthood and the production of first egg sacs by females in each treatment (10 nauplii each in 5 replicates), the number of adults in each replicate was reduced to two pairs of adults (2 males and 2 females). Where possible, the appropriate

numbers of founder male and females were selected from the same replicate. If this was not possible (e.g. because of limited development in the low food concentrations), founder pairs were constructed from different replicates. If insufficient adult copepods were available in each replicate to provide 2 pairs, replicates were started with one male and one female (Table 7.1). As the objective was to compare population growth in the different treatments, it was deemed important to begin experiments with the same number (and sex ratio) of copepods per treatment. Observations of mortality were made at 24 h intervals and the surviving copepods transferred to duplicate test chambers containing freshly prepared test solutions. Experiments were continued for 77 days and the numbers of copepods in each test chamber were measured once per week during the first 28 days and twice per week (intervals of 3 and 4 days) thereafter. Population measurements provided a quantitative assessment of both the total number of copepods present and the proportion of different life stages that constituted the whole population. The proportion of different life stages was measured by recording the number of copepods in four categories: nauplii, small copepodids, large copepodids and adults, and egg sac females. The total numbers of nauplii, and egg sac females are explicit but accurate discrimination in numbers between the different categories for copepodids and adults was more difficult. Consequently, these numbers must be considered an approximation although the data do provide an indication of the progress of development of the copepodid to the adult stage. Whilst it is interesting to define the number of different life stages present in the population at any one time, biomass rather than number alone, may be a more important determinant of population growth under the different test conditions. Biomass measurements were not made directly as the populations could not be sacrificed for weighing, however, the dry weight data provided by Heath (1994) for different life stages of the sibling species *Tisbe holothuriae* were used to provide an estimate of changes in biomass with time. It is important to note that this procedure assumes no response in weight to either PCP or food limitation.

These assumptions were not validated, however, PCP and/or food limitation may have a negative response on weight and, therefore, results may underestimate effects. Body size (length) of male and female copepods was measured in previous experiments (Chapter 6; Table 6.7) but limited data prevented statistical comparisons of length data between control and PCP treatments and those fed low and high algal food concentrations (1300 and 3250 $\mu gC\ l^{-1}$). For each of the four different categories of life stages used to monitor changes in population growth with time (nauplii, small copepodids, large copepodids and adults and ovigerous females), a dry weight value was assigned as shown in Table 7.2. Consequently, population measurements based on the numbers of copepods in each category at different time intervals were also translated into an estimate of biomass using the average values denoted in Table 7.2. Temperature (daily) and the pH and dissolved oxygen concentration (at least weekly) was measured in each treatment.

7.2.2. Statistical analysis

The sex ratio of adult copepods was examined using a contingency table procedure (Hayslett & Murphy, 1976) to identify significant departures from a sex ratio of 1:1. The data for time to first egg sac and population number and biomass were tested for normality and equality of variances prior to analysis of variance techniques (Sokal & Rohlf, 1995). Data that failed to meet the assumptions for analysis of variance were analysed using non-parametric (distribution-free) techniques (Steel, 1959; Hollander & Wolfe, 1973). Pairwise comparisons between corresponding control and PCP treatments for time to first egg sac were made by Student's *t*-test (two-tailed). Within each diet, differences in time to first egg sac between different algal concentrations were analysed using 1-way ANOVA followed by *t*-tests (two-tailed) to identify significant differences between the treatment means.

7.3. RESULTS

7.3.1. Development from hatching to production of the first egg sac

The effects of PCP and algal diet on copepod survival, sex ratio, the number of fertile and infertile females and the time taken for females to produce their first egg sac are summarised in Table 7.3. In the solvent control, significant copepod mortality was observed at food concentrations of 520 μgC l^{-1}, but not 1300 and 3250 μgC l^{-1}, for both the unialgal and mixed species algal diet (Table 7.3). Compared with the corresponding control, exposure to 100 μg l^{-1} PCP resulted in significant mortality of the copepods, irrespective of food quality (unialgal versus mixed algal diet) and quantity (Table 7.3, Fig. 7.1).

For the mixed species algal diet, a reduction in algal concentration from 3250 to 1300 and 520 μgC l^{-1} resulted in a significant deviation in sex ratio from 1:1 (Table 7.3). Sex ratios did not depart significantly from 1:1 for control copepods fed on the unialgal diet (Table 7.3). There were no significant changes in the sex ratio of copepods exposed to PCP treatments, however, these results must be treated with caution. Where mortality had occurred, and copepods could be sexed, males nearly always outnumbered females and a high incidence of male mortality (males may be more sensitive to PCP than females) may have biased the results of the sex ratio for surviving copepods (Table 7.3).

The time to first egg sac for surviving females in the PCP treatments was compared with the corresponding values for females in the solvent control (Fig. 7.2). Compared with the controls, surviving females in the PCP treatments took longer to produce their first egg sac indicating a delay in reproduction. These differences were not significant at the lowest ration (520 μgC l^{-1}), however, these results should be treated cautiously due to the small numbers of surviving copepods available for analysis. By comparison, differences between control and PCP treatments were significant for copepods fed 1300 or 3250 μgC l^{-1} of the unialgal diet but

only for 1300 μgC l^{-1} for those fed a mixed species algal diet. These results suggest that the effect of PCP on the time to first egg sac may have been influenced by the type of algal diet.

In summary, the results from the first stage of these population experiments showed high mortality of the early life stages in 100 μg l^{-1} PCP treatments compared with the control. Furthermore, of the surviving copepods that developed to the adult stage, there was a delay in the onset of reproduction by females in the PCP treatments and these results appeared to be influenced by the type of algal diet used (i.e. food quality).

7.3.2. Population experiments

Population measurements (number and biomass) of copepods exposed for 11 weeks in control and PCP treatments receiving 3250, 1300 and 520 μgC l^{-1} of either a unialgal or mixed species algal diet are summarised in Tables 7.4 - 7.6 respectively. Copepods fed either unialgal or mixed species algal diets could not be sustained at 520 μgC l^{-1} (Table 7.6). In these treatments, copepod numbers remained small, gradually declining until the populations became extinct, or nearly so, after 63 - 77 days. Population numbers of copepods fed 1300 or 3250 μgC l^{-1} of a unialgal or mixed species algal diet increased during the first 21 days, thereafter remained relatively stable, albeit with relatively small oscillations of increase or decrease in numbers (Figs 7.3 and 7.4). Estimates of biomass (μg dry weight) showed the same trend (Figs 7.5 and 7.6), although oscillations generally tended to be less pronounced. In view of the relatively large variation in size between the different life-history stages, measurements of population size based solely on numbers may be misleading. Large numbers of nauplii may contribute significantly to changes in population number but, because of their small size, may contribute little to changes in biomass. For the purposes of this study, nauplii, small copepodid, and large copepodid and adults were selected as three distinct age classes (Section 7.2.1). By measuring the proportion of copepods in these age classes, it was possible to follow

the production of nauplii and their subsequent growth and development into the copepodid and adult components of the population. For example, the data from control and PCP-exposed populations receiving 3250 $\mu gC\ l^{-1}$ of a unialgal or mixed species algal diet revealed cycles of nauplii production and their contribution to total population numbers (Figs 7.7 and 7.8 respectively). Translating population numbers into estimates of biomass revealed that biomass was eventually dominated by the large copepodid and adult stages. For biomass, nauplii and small copepodids provided a smaller contribution to population size compared with copepod numbers.

There were clear indications of differences in population size during the early phase of exposure, and statistical analysis of day 14 and 21 day data (population number and biomass) revealed consistent and significant reductions in population size between control and PCP-exposed populations, irrespective of unialgal or mixed species algal diet (Table 7.7). At or beyond day 28, populations appeared to reach steady state, or carrying capacity, showing relatively small fluctuations in population size. During this period (day 28 - 77), statistical analysis revealed a limited number of occasions when differences in population number and biomass between PCP and control populations were significant (Table 7.7).

To discern the main trends in population growth during the period of reaching their carrying capacity, the measurements of population number and biomass during weeks 4 - 11 (n=8) were translated into an overall mean value (Table 7.8 and Fig. 7.9). These data could not be statistically analysed, therefore, it could not be ascertained whether differences in carrying capacity due to changes in food quantity or food quality (unialgal versus mixed algal diets) were significant. Results showed that food quantity and quality had an important influence on the carrying capacity of the population. Irrespective of whether copepods were fed a unialgal or mixed species diet, there was a consistent reduction in the carrying capacity of populations (numbers and biomass) in control and PCP treatments following a reduction in

algal concentration from 3250 to 1300 µgC l^{-1} (Table 7.8). The decline in population biomass following a reduction in algal concentration is shown in Figures 7.10 and 7.11 respectively for treatments receiving the unialgal and mixed species algal diet. In relation to food quality, control and PCP-exposed populations receiving 1300 or 3250 µgC l^{-1} of a mixed species algal diet achieved a higher carrying capacity (numbers and biomass) than corresponding treatments receiving a unialgal diet (Table 7.8). The results describing the effect of algal concentration and diet type on the population response were also presented visually to confirm the trends in these parameters described above. Results for control and PCP treatments receiving unialgal and mixed diets were expressed as a ratio of response at low and high ration (1300/3250 µgC l^{-1}). Results clearly show a reduction in biomass at the lower ration but also indicate that control and PCP populations respond similarly to a reduction in algal concentration from 3250 to 1300 µgC l^{-1} (Fig. 7.12). Similarly, the effect of algal diet on population growth in control and PCP treatments was presented as a ratio of response to unialgal and mixed species algal diet (unialgal diet/mixed species diet) at 1300 and 3250 µgC l^{-1}. Results confirm a reduction in final biomass for copepods receiving the unialgal diet, as opposed to the mixed species algal diet (Fig. 7.13).

Differences in carrying capacity (numbers and biomass) between control and PCP-exposed populations were relatively small at algal concentrations of 1300 µgC l^{-1} (0.9 - 8.4%) and 3250 µgC l^{-1} (18 - 28%) and it is not known whether these differences are statistically significant. To facilitate comparison of population differences between PCP and control treatments, results for copepod biomass were expressed as a ratio of PCP to control biomass (Table 7.7). Ratios < 1 indicate a reduction in population biomass and ratios > 1, an increase in population biomass, compared with the control. At the high ration mixed species algal diet (3250 µgC l^{-1} of *Isochrysis* and *Rhodomonas*), copepod biomass was consistently lower for populations exposed to PCP, and values remained relatively stable from day 28 - 77 (Fig.

7.14). A different response was observed for copepods receiving a high ration unialgal diet (3250 μgC l^{-1} of *Isochrysis*) and copepod biomass remained generally higher in PCP than in control populations. By comparison, the growth of populations receiving 1300 μgC l^{-1} (2.5 times less food) appeared less stable and biomass in PCP treatments fluctuated between periods of increase and decrease relative to the control (Fig. 7.14).

7.4. DISCUSSION

Studies of harpacticoid population growth have focused more on the development of mass rearing techniques for aquaculture rather than on the effect of chemicals. Consequently, limited information is available concerning the potential effects of chemical contaminants on harpacticoid populations. Hoppenheit (1977), Hoppenheit and Sperling (1977) and Brand *et al.* (1986) described reductions in copepod numbers for populations of *Tisbe holothuriae* exposed to cadmium. Additional data describing the effect of chemicals on populations growth are available for freshwater cladocerans. Munzinger (1994) reported the effects of nickel on populations of the freshwater cladoceran *Daphnia magna* and Van Leeuwen *et al.* (1987) reported significant effects on the yield, or carrying capacity, of *D. magna* populations exposed to ten different chemicals.

Results for *Tisbe battagliai* indicate PCP causes significant deleterious effects on population growth during the first 21 days but, thereafter (days 28 - 77), population densities appeared to be relatively stable (i.e. once the carrying capacity was achieved). Between days 28 - 77, there were some significant PCP-related effects but no clear trends were identified (Table 7.7). Several factors may have contributed to the apparent 'recovery' of PCP-exposed populations after 21 days. Considering the relatively high mortality (18 - 90%) of the early life stages in PCP treatments during development to the adult stage, it may be argued that

subsequent selection of surviving adults to start founder populations was biased towards those individuals which were more resistant to PCP (selection of resistant genotypes). Nevertheless, there was a significant reduction in population size of PCP treatments compared with the corresponding control during the first 21 days and the effects of PCP were attributed to reductions in offspring production (effects on the F1 generation). Data on the stage structure of populations receiving the high ration diet (Figs 7.7 and 7.8) indicate that the F1 generation reached the adult stage and produced offspring (F2 generation) during the 77 day exposure period. Chemicals may play an important role in natural selection amongst aquatic organisms, particularly when conditions involve long-term exposure to sublethal concentrations. Information on the responses of life-history traits during selection to chemicals is scarce, however, the development of PCP tolerance by *Tisbe* must be considered as one possible explanation to describe the apparent recovery of PCP-exposed populations after 21 days. Given the relatively short time period for recovery to occur, physiological acclimation, rather than genetic adaptation of organisms may be the most likely mechanism involved. Selection experiments in the laboratory have revealed that an increased tolerance to metals may evolve within a few generations of *Tisbe holothuriae* (Moraitou-Apostolopoulou *et al.*, 1983). By comparison, long-term exposure of the chironomid *Chironomus riparius* during nine consecutive generations (about 1 year) revealed that low, environmentally realistic, concentrations of cadmium modified the normal fluctuations in population growth rates (Postma & Davids, 1995). These authors revealed that the effects of cadmium on mortality, growth and reproduction increased during consecutive generations but decreasing effects on growth and reproduction were observed towards the end of the experiment, indicating an increased tolerance to cadmium (Postma & Davids, 1995). It would appear that the development of tolerance in aquatic organisms may cause changes in population dynamics but such responses are likely to be both species and chemical specific.

Experimental conditions did not exclude microbial populations and, during the exposure period, the possible development of a microbial population adapted to, and subsequently able to biodegrade, PCP may have reduced the exposure concentration with time. Chemical analysis of the test solutions would be required to confirm whether the PCP exposure concentrations remained close to nominal.

Population density was influenced by food concentration and diet type (unialgal or mixed species diet). These results were consistent with those from previous experiments which showed significant reductions in fecundity of *Tisbe battagliai* following relatively small reductions in algal concentration (Chapters 3 and 4) and significant increases in fecundity for copepods fed a mixed species algal diet compared with a unialgal diet of *Isochrysis galbana* (Chapters 5 and 6). Previous experiments revealed prolonged stage durations and reduced fecundity for individual copepods receiving 520 μgC l^{-1} of a unialgal diet (Chapters 2 and 3 respectively) indicating that this concentration may be a threshold food concentration for development and reproduction. Results from the present experiments tend to support this latter suggestion. Control and PCP-exposed populations receiving 520 μgC l^{-1} of a mixed species or unialgal diet became extinct, or nearly so, after 77 days, indicating that the potential improvements in food quality derived from a mixed species algal diet provided no additional benefit. By comparison, copepods receiving a higher algal ration (1300 or 3250 μgC l^{-1}) of the mixed species algal diet attained higher population densities than those receiving a unialgal diet of *Isochrysis galbana*. The phytal biotope occupied by some species of *Tisbe*, offers a potentially abundant supply of food for phytal harpacticoids (Hicks, 1977, 1979). The apparent sensitivity of individuals and populations of *Tisbe battagliai* to relatively small reductions in food concentration below optimal levels for development and reproduction may provide supporting evidence for the evolution of life-history traits in environments (the phytal biotope) where food resources are not limiting.

In these *Tisbe* experiments, the control populations expanded during the first 21 days as a result of the production of nauplii (F1 generation) by the founder females. Populations were growing in a limited space and the density gradually rose until increasing competition for food resources and space, reduced the rate of increase of the population until eventually the population ceased to grow, or remained within a narrow range of densities. According to Begon *et al.* (1990), intraspecific competition, by acting on birth and death rates, can regulate populations at a stable density at which the birth rate equals death rate. This density is known as the carrying capacity of the population and it represents the population size which the resources of the environment can just maintain ('carry') without a tendency to either increase or decrease. For populations of *Tisbe*, the results based on estimates of biomass present a different perspective to those for copepod numbers (Figs 7.7 and 7.8). Although nauplii and small copepodid stages may be present in the population in relatively large numbers, they are much smaller in size compared with the adult stages (Table 7.2) and contribute less to the total population biomass.

In the current experiment, populations of *T. battagliai* reached a maximum stable density of approximately 11 copepods per ml^{-1} on the high ration (3250 $\mu gC\ l^{-1}$) mixed species algal diet. This value was compared with published data on the maximum population density of harpacticoids achieved in laboratory culture. Irrespective of copepod inoculum density, populations of *T. holothuriae* receiving a high ration mixed diet of algae and an inert food, levelled out at between 10 - 20 per ml^{-1} (Heath, 1994). Similar values were obtained by Gaudy and Guerin (1982) indicating, perhaps, that these numbers represent an upper threshold for population density in *T. holothuriae*. Higher population densities (33 per ml^{-1}) had a deleterious effect on the growth rate and sex ratio of *T. holothuriae* but no link was found between a build up of metabolites in the culture water or inbreeding depression and population crashes (Heath, 1994). Instead, population crashes in mass cultures were attributed to

competition for space which resulted in increased swimming activity (with associated increase in energetic costs) and reduced productivity. For benthic surface dwelling organisms such as *Tisbe*, the surface area of culture and test vessels may be an important determinant of final population density. The observed differences in carrying capacity due to diet composition (food quality) in the present population experiments suggest that factors other than, or in addition to, competition for free space, influence final population size of *T. battagliai.*

Data from laboratory population experiments with *Tisbe battagliai* present evidence that intraspecific competition tends to regulate population size within relatively narrow limits. Self-regulating mechanisms can be explained most easily by individual selection operating on mechanisms of interference competition within a species (Krebs, 1994). Natural selection will favour individuals that increase their fitness by means of higher reproduction and lower mortality but it will also favour individuals that reduce the fitness of their neighbours by any technique of interference competition. Several mechanisms have been described for density-dependent control of population size in the *Tisbe* genus and general discussion has focused on the mechanisms whereby animals respond to crowding. Studies of *Tisbe holothuriae* have reported reductions in reproductive output and viability at high population densities (Hoppenheit, 1975ab, 1976). Fava and Crotti (1979) reported negative effects of crowding on nauplii production by *T. holothuriae* but found that these effects diminished when the water medium was renewed daily. These authors concluded that a chemically-mediated mechanism was the most likely explanation for reduced fertility with increased crowding. So far, these studies (Hoppenheit, 1975ab, 1976; Fava & Crotti, 1979) describe potential interactions between adults but there is evidence that nauplii of *T. holothuriae* also possess mechanisms of inhibition. Brand (1985) found that crowding of *T. holothuriae* depressed larval viability as a result of behavioural changes which reduced contact with the food. Brand (1985) hypothesised that larval inhibition could have evolved as a mechanism for staggering larval

development or as a means of reducing the fitness of neighbouring individuals. Kahan *et al.* (1988) reported maternal inhibition of hatching at high population densities of the harpacticoid *Tigriopus japonicus* finding that high densities of egg-carrying females, or conditioned medium from dense cultures, inhibited hatching although effects diminished considerably after 48 h. Electron microscopy revealed a physical structure between mother and egg sac that may have been responsible for transmission of the inhibitory message (Kahan *et al.*, 1988). Literature data show that the sex ratio in *Tisbe* is strongly shifted in favour of males in high density cultures but biased towards females at low population densities (Hoppenheit, 1976; Uhlig, 1984; Heath, 1994). Changes in sex ratio towards males at high density, and subsequent reduction in the reproductive potential of the population, may act as a possible negative feedback mechanism for controlling population density. It is not known whether the different mechanisms described above, operate individually or in concert, but they appear to be effective in regulating population density of *Tisbe* to within relatively narrow limits (stable population).

It is not known whether the densities of *Tisbe* obtained in laboratory culture are attained in the field. Close proximity of the adult sexes may facilitate reproduction and adults and nauplii may congregate in numbers to exploit food resources but it is not known whether the different life stages occur in close proximity or occupy different niches. Unlike laboratory experiments, where population size is eventually limited by space (vessel size), harpacticoids are not similarly constrained in the field. Limited information is available concerning the population dynamics of *Tisbe* under field conditions. Lopez (1982) observed cycles in local population abundance of *Tisbe cucumariae* at the site of dead macroinvertebrates (tunicates). Experiments were conducted in a large flowing seawater holding tank (3.5 m deep and 10 m in diameter) considered to represent field conditions and, because of the large enclosure, highlighted aspects of behaviour that would be difficult to reproduce in smaller test vessels. The corpses of tunicates were rapidly colonised by adults, followed by a period of intense

reproduction (bloom phase). Due to the cyclic recruitment of nauplii, a step-wise increase in the abundance of adults was expected but did not happen. Data suggested that adults were removed from the population in some density-dependent manner, either through adult mortality, emigration, or both. After 23 days of large changes, the life-history stage structure of the population at the dead tunicate became relatively stable and consisted largely of adults. The author speculated on reasons for the apparent disappearance of nauplii after 23 days, suggesting naupliar mortality due to starvation, and a reduction in the development rate of nauplii (to avoid over exploitation of localised food resources), as possible causes (Lopez, 1982). Bergmans (1983) considered that density effects in *Tisbe* are most likely an effect of adults on juvenile survival and at high population densities, nauplii may be more sensitive than adults to crowding. Results from previous experiments (Chapters 2 and 3) suggest that adult *Tisbe battagliai* have a higher tolerance to low food concentration than the early life-history stages. Similar observations have been reported for freshwater calanoids (Jamieson & Burns, 1988). Therefore, under conditions of food limitation, leading to temporal reduction of food at high copepod density, it may be expected that the larger life stages (large copepodids and adults) are able to persist at lower food levels than the smaller life stages. Munzinger (1994) observed that high animal density in populations of *Daphnia magna* led to a shortage of available food per animal and, as neonates are very sensitive to starvation, they died. Only when most of the adults had died, were offspring able to reach sexual maturity and produce offspring, size classes returning to a predominance of adults when food became limiting again. Despite the broad differences in experimental design, the results obtained by Lopez (1982) indicating the attainment of a stable age structure consisting mostly of adults is in agreement with the population data for *Tisbe battagliai.* Lopez (1982) concluded that local cycling of numbers in *T. cucumariae* populations was mediated by immigration of adults and late copepodid stages, local reproduction and emigration. In the laboratory, practical reasons

dictate the use of relatively small test volumes. Small test volumes have advantages in that populations are able to reach carrying capacity relatively quickly thereby permitting the operator to examine the effect of chemicals through population cycles of exponential growth and the conditions leading to intraspecific competition and population regulation. Unfortunately, key processes such as immigration and emigration of individuals are difficult to represent in the laboratory, therefore, these aspects of population dynamics are rarely considered.

Lopez (1982) advised caution when drawing conclusions from demographic data obtained in the laboratory, pointing out that observations and experiments may be influenced by laboratory artefacts. Such concerns are valid, and some of the limitations associated with laboratory culture and testing of harpacticoid copepods (e.g. inbreeding depression and changes in sex ratio) have been discussed in detail elsewhere (Chapter 4). In relation to food quantity and quality, population experiments with *Tisbe battagliai* confirmed the trends identified from experimental approaches based on the measurement of individual copepods (Chapters 2 to 6). Measurement of individual copepods revealed the importance of food concentration, and its associated nutritional quality, for the population dynamics of *Tisbe* and these effects were manifest in population experiments. The results describing the effects of PCP, and its potential interaction with a key environmental variable (food quality and quantity) at the individual and population level for *T. battagliai*, revealed some important differences between the individual and population approaches. Unlike traditional toxicity experiments, where experiments are performed using a range of test concentrations, the experimental design used in population experiments used a single exposure concentration of PCP (100 $\mu g\ l^{-1}$). This concentration of PCP, dependent on the influence of environmental factors (e.g. food quantity and quality), represents a threshold concentration for chronic toxicity to *Tisbe* (Chapter 6), therefore, the results from these experiments were anticipated to reveal subtle effects of the

chemical and its interaction with the test diets. In previous experiments, the effects of PCP were dependent on food concentration and significant reductions in offspring production, and decreases in r_m , were observed at 1300 µgC l^{-1} but not at 3250 µgC l^{-1} (Chapter 6). In the present experiments, exposure of nauplii to PCP resulted in significant mortality and a delay in the time to production of the first egg sac by surviving females. Subsequent increases in the generation time, and reductions in offspring production, resulted in a significant reduction in initial population growth in the PCP treatments compared with the control during the first 21 days. Laboratory populations of *Tisbe battagliai* appeared to follow logistic type growth hence the populations rate of increase r_m will affect the initial phase of population growth, but not the carrying capacity, which is determined by food concentration (Krebs, 1994). The outcome was a reduction in initial population growth in the PCP treatments compared with the control during the first 21 days. Differences in population size between control and PCP treatments were not apparent after 28 days (carrying capacity) and potential reasons to explain these differences were put forward earlier in this discussion.

Table 7.1. Creation of founder populations. Values denote the number of male (m) and female (f) copepods used to start the founder population and the day (after hatching) upon which the population was established (* Note that 1 male and 1 female were added on day 14, another pair of adults on day 18).

Algal conc. ($\mu gC\ l^{-1}$)	Rep	Solvent control		100 $\mu g\ l^{-1}$ PCP	
		Isochrysis + *Rhodomonas*	*Isochrysis*	*Isochrysis* + *Rhodomonas*	*Isochrysis*
3250	1	2m2f day 11	2m2f day 11	2m2f day 13	2m2f day 11
	2	2m2f day 11	2m2f day 11	0m2f day 13	2m2f day 13
	3	2m2f day 11	2m2f day 11	0m2f day 12	2m2f day 13
	4	2m2f day 11	2m2f day 11	2m2f day 12	2m2f day 11
	5	2m2f day 11	2m2f day 11	2m2f day 14	2m2f day 11
1300	1	2m2f day 14	2m2f day 13	1m2f day 19	-
	2	2m2f day 12	2m2f day 14	1m1f day 19	-
	3	2m2f day 13	2m2f day 12	1m2f day 17	1m2f day 17
	4	2m2f day 13	2m2f day 12	0m2f day 19	-
	5	2m2f day 17	2m2f day 13	2m2f day 18	0m2f day 18
520	1	-	2m2f day 18	1m1f day 20	-
	2	-	-	1m2f day 18	-
	3	2m2f day 20	2m2f day 19	-	2m2f day 20
	4	2m2f day 19	2m2f day 14*	-	-
	5	2m2f day 21	-	-	-

Table 7.2. Dry weight data for different life stages of *Tisbe holothuriae*. Data provided by Heath (1994).

Lifestage	Dry weight (μg)	Average weight (μg)	Corresponds to category
N1-N2	0.05	0.07	Nauplius
N5	0.09		
C1	0.15	0.27	Small copepodid
C3	0.38		
C4	0.82	1.23	Large copepodid and
Male	0.96		adult
Female	1.91		
Ovigerous female	4.92	4.92	Ovigerous female

Table 7.3. Development from hatching to production of the first egg sac. Experimental design consisted of 5 replicates, each containing 10 nauplii. Mortality was generally higher for males than for females, therefore, in those PCP treatments where a relatively large number of copepods died before sexing, a high incidence of male mortality in the unsexed animals could have biased the results of the sex ratio observed in surviving copepods [† indicates a significant ($P < 0.05$) departure in sex ratio from 1:1; [a] indicates significant ($P < 0.05$) mortality compared with the highest algal concentration; [b] indicates significant ($P < 0.05$) mortality compared with the corresponding control; * indicates a significant ($P < 0.05$) difference between the control and corresponding PCP treatment]. Algal diets consist of *Isochrysis galbana* (I) and a mixture of *I. galbana* and *Rhodomonas reticulata (I + R).*

Test conc. ($\mu g\ l^{-1}$)	Algal diet	Algal conc. ($\mu gC\ l^{-1}$)	Sex ratio			Mortality				Female development		
			no. male	no. female	% male	no. unsexed	no. male	no. female	% total	no. + egg sac	no. - egg sac	day of first egg sac (mean ± 1SD)
Solvent	I + R	3250	25	25	50	0	0	0	0	25	0	11.1 ±1.39
control		1300	35	15	70†	0	3	1	8	14	0	13.1 ±1.69*
		520	44	6	88†	0	17	1	36[a]	5	1	17.2 ±1.79
	I	3250	23	27	46	0	0	0	0	27	0	12.0 ±2.64*
		1300	25	25	50	0	0	0	0	25	0	14.6 ±2.81*
		520	31	19	62	0	6	8	28[a]	6	5	19.7 ±0.52
100 $\mu g\ l^{-1}$	I + R	3250	27	21	56	2	6	1	18[b]	20	0	11.9 ±1.39
PCP		1300	22	13	63	15	19	5	78[b]	4	4	17.0 ±0.82
		520	15	6	72	29	10	2	82[b]	2	2	19.5 ±0.71
	I	3250	12	20	38	18	4	2	48[b]	17	1	14.0 ±2.42
		1300	16	18	47	16	12	7	70[b]	11	0	17.0 ±2.53
		520	10	6	63	34	8	3	90[b]	3	0	18.3 ±0.58

Table 7.4. Population growth of *Tisbe battagliai* fed 3250 µgC l^{-1} of a unialgal (*Isochrysis galbana*) and mixed species algal diet (*I. galbana* and *Rhodomonas reticulata*) and exposed to 0 (solvent control) and 100 µg l^{-1} PCP. Values are expressed as the mean ±1SD of the number or biomass (µg dry weight) of copepods present in a volume of 10 ml [* indicates a significant ($P < 0.05$) difference in population size between control and PCP treatment].

Algal diet	Test day	Population number		Population biomass	
		Control	PCP	Control	PCP
Isochrysis	14	59 ±19	13 ±09*	13.2 ±3.07	6.60 ±2.27*
	21	153 ±25	53 ±33*	61.5 ±7.69	17.0 ±6.91*
	28	52 ±20	46 ±28	27.5 ±10.0	35.5 ±7.52
	35	36 ±15	64 ±39	43.4 ±12.6	62.6 ±19.4
	42	40 ±31	50 ±17	31.1 ±21.7	38.0 ±4.96
	49	39 ±27	61 ±27	35.4 ±15.4	55.2 ±15.1*
	56	32 ±14	42 ±24	29.3 ±10.1	41.3 ±13.8
	63	43 ±09	32 ±18	31.2 ±11.6	26.9 ±13.0
	70	58 ±57	53 ±14	35.7 ±18.2	36.2 ±9.51
	77	31 ±28	51 ±14	28.4 ±15.2	40.7 ±11.6
Isochrysis +	14	77 ±15	29 ±09*	17.4 ±1.06	5.36 ±1.41*
Rhodomonas	21	174 ±48	93 ±33*	82.5 ±10.4	26.7 ±6.83*
	28	68 ±25	67 ±19	72.6 ±16.1	52.5 ±10.0
	35	126 ±57	85 ±26	78.2 ±16.4	56.6 ±6.15*
	42	115 ±52	100 ±42	91.1 ±24.0	69.9 ±16.3
	49	140 ±31	133 ±62	84.1 ±15.9	69.7 ±11.0
	56	115 ±48	78 ±23	60.7 ±4.24	58.7 ±2.42
	63	78 ±21	40 ±12	67.5 ±9.71	44.7 ±14.2*
	70	88 ±48	106 ±38	72.1 ±9.89	63.7 ±9.54
	77	149 ±66	112 ±13	72.8 ±15.7	69.1 ±7.08

Table 7.5. Population growth of *Tisbe battagliai* fed 1300 µgC l^{-1} of a unialgal (*Isochrysis galbana*) and mixed species algal diet (*I. galbana* and *Rhodomonas reticulata*) and exposed to 0 (solvent control) and 100 µg l^{-1} PCP. Values are expressed as the mean ±1SD of the number or biomass (µg dry weight) of copepods present in a volume of 10 ml [* indicates a significant ($P < 0.05$) difference in population size between control and PCP treatment]. Zero values indicate that founder populations were not established at this time.

Algal diet	Test day	Population number		Population biomass	
		Control	PCP	Control	PCP
Isochrysis	14	16 ±24	0	11.7 ±1.64	0
	21	51 ±04	20 ±18*	20.6 ±6.44	8.66 ±9.51*
	28	25 ±10	46 ±25	14.4 ±6.86	24.4 ±6.75*
	35	14 ±08	36 ±15*	18.4 ±6.45	33.3 ±7.99*
	42	34 ±19	28 ±13	20.1 ±5.36	22.3 ±10.8
	49	52 ±23	27 ±10*	23.2 ±9.38	28.9 ±18.3
	56	31 ±15	34 ±16	16.6 ±4.79	17.4 ±5.16
	63	19 ±10	09 ±02*	17.5 ±7.73	7.52 ±2.89*
	70	23 ±12	10 ±05*	16.8 ±9.12	10.8 ±2.77
	77	41 ±25	29 ±11	22.9 ±7.59	13.1 ±1.30
Isochrysis +	14	0	0	0	0
Rhodomonas	21	72 ±34	49 ±05	25.7 ±9.90	9.99 ±0.47*
	28	55 ±19	57 ±23	33.1 ±11.2	30.2 ±0.30
	35	65 ±53	35 ±12	35.8 ±13.2	30.9 ±9.36
	42	66 ±23	39 ±01	31.5 ±13.3	34.6 ±1.69
	49	87 ±37	122 ±51	32.6 ±12.6	49.7 ±11.2
	56	45 ±15	55 ±16	21.2 ±5.43	23.8 ±0.42
	63	25 ±06	25 ±04	22.9 ±4.86	20.1 ±7.41
	70	31 ±14	48 ±43	23.1 ±1.47	30.4 ±3.02*
	77	66 ±15	63 ±18	28.3 ±3.11	20.6 ±2.45*

Table 7.6. Population growth of *Tisbe battagliai* fed 520 µgC l^{-1} of a unialgal (*Isochrysis galbana*) and mixed species algal diet (*I. galbana* and *Rhodomonas reticulata*) and exposed to 0 (solvent control) and 100 µg l^{-1} PCP. Values are expressed as the mean ±1SD of the number or biomass (µg dry weight) of copepods present in a volume of 10 ml. Zero values indicate that founder populations were not established at this time and - indicates that the population became extinct (no survivors in any replicate)

Algal diet	Test day	Population number		Population biomass	
		Control	PCP	Control	PCP
Isochrysis	14	0	0	0	0
	21	04 ±0	0	9.84 ±4.26	0
	28	24 ±17	42 ±0	10.9 ±4.61	17.7 ±0
	35	22 ±12	46 ±0	9.78 ±0.77	15.1 ±0
	42	03 ±01	02 ±0	4.10 ±0.71	0.54 ±0
	49	02 ±01	02 ±0	2.87 ±0.71	2.46 ±0
	56	02 ±01	02 ±0	2.05 ±0.71	2.46 ±0
	63	-	-	-	-
	70	-	-	-	-
	77	-	-	-	-
Isochrysis +	14	0	0	0	0
Rhodomonas	21	19 ±14	08 ±0	7.11 ±3.38	2.88 ±0
	28	36 ±27	18 ±0	14.4 ±3.82	13.4 ±0
	35	20 ±13	10 ±0	14.6 ±8.70	6.50 ±0
	42	09 ±08	01 ±0	6.35 ±5.79	0.27 ±0
	49	05 ±08	01 ±0	4.69 ±5.60	1.23 ±0
	56	02 ±02	-	2.96 ±4.11	-
	63	01 ±02	-	0.93 ±0.83	-
	70	0.3 ±0.6	-	0.41 ±0.71	-
	77	0.3 ±0.6	-	0.41 ±0.71	-

Table 7.7. Differences in population size (number and biomass of copepods per ml^{-1}) between corresponding control and PCP treatments. Data are expressed as an effect ratio (PCP/control), ratios < 1 indicate a reduction in population size and ratios > 1, an increase in population size, compared with the control [* indicates a significant difference ($P < 0.05$) in population size between control and PCP treatment, values taken from Tables 7.4 and 7.5 respectively].

Test day	3250 µgC l^{-1} *Isochrysis + Rhodomonas*		3250 µgC l^{-1} *Isochrysis*		1300 µgC l^{-1} *Isochrysis + Rhodomonas*		1300 µgC l^{-1} *Isochrysis*	
	Number	Biomass	Number	Biomass	Number	Biomass	Number	Biomass
14	0.38*	0.31*	0.22*	0.50*	0	0	0	0
21	0.53*	0.32*	0.35*	0.28*	0.68	0.39*	0.39*	0.42*
28	0.99	0.72	0.88	1.29	1.04	0.91	1.84	1.69*
35	0.67	0.72*	1.78	1.44	0.54	0.86	2.57*	1.81*
42	0.87	0.77	1.25	1.22	0.59	1.10	0.82	1.11
49	0.95	0.83	1.56	1.56*	1.40	1.52	0.52*	1.25
56	0.68	0.97	1.31	1.41	1.22	1.12	1.10	1.05
63	0.51	0.66*	0.74	0.86	1.00	0.88	0.47*	0.43*
70	1.20	0.88	0.91	1.01	1.55	1.32*	0.43*	0.64
77	0.75	0.95	1.65	1.43	0.95	0.72*	0.70	0.57
Overall mean (weeks 4 - 11)	0.83	1.06	1.26	1.28	1.04	1.05	1.06	1.07

Table 7.8. Overall mean values (n=8) for population size (number and biomass) during weeks 4 - 11 (days 28 - 77), the period during which population size had appeared to reach a stable density (carrying capacity) for each population. Results are expressed as the % increase or decrease in population size following exposure to PCP, and the % increase in population size for copepods fed a mixed species of *Isochrysis* and *Rhodomonas* (I+R) instead of a unialgal diet of *Isochrysis* (I).

Algal diet	Population number (per ml^{-1})			Population biomass ($\mu g\ ml^{-1}$)		
	Control	PCP	% change	Control	PCP	% change
3250 I+R	10.99	9.01	-18	7.49	6.06	-19
3250 I	4.14	4.99	+21	3.28	4.21	+28
% change	+165	+81	-	+128	+44	-
1300 I+R	5.50	5.55	+0.9	2.86	3.00	+4.9
1300 I	2.99	2.74	-8.4	1.87	1.97	+5.3
% change	+84	+103	-	+53	+52	-

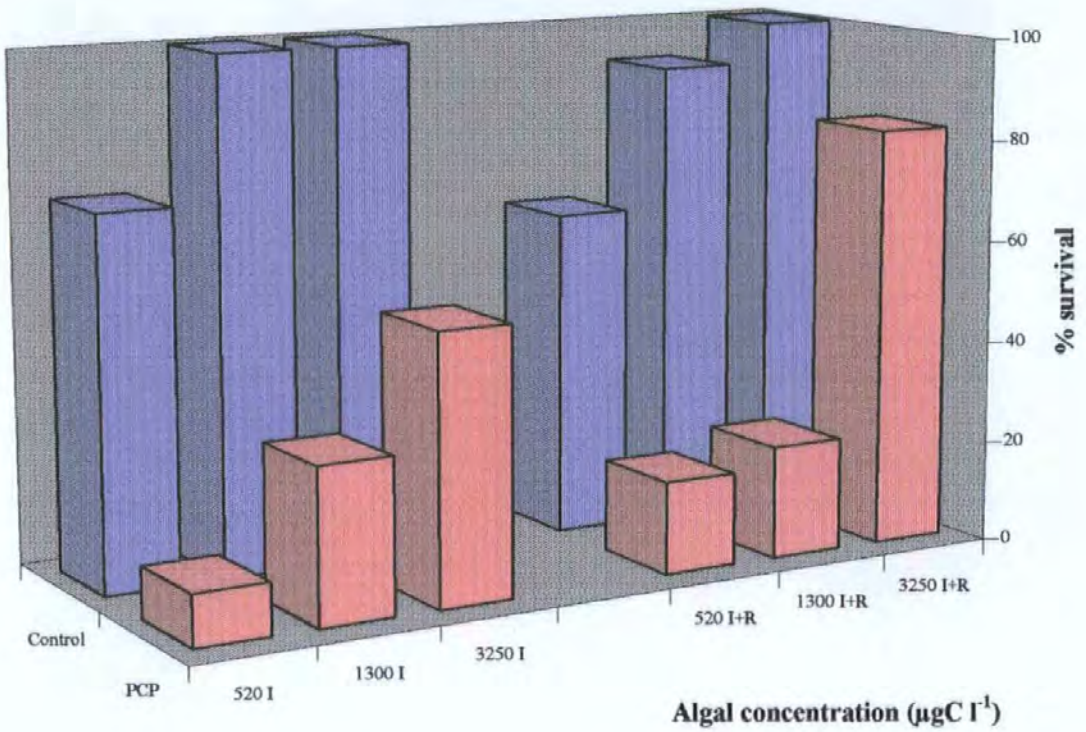


Figure 7.1. The effect of PCP (100 µg l), and algal concentration (520, 1300 and 3250 µgC l) on the cohort survival of *Tisbe battagliai* in population experiments. Algal diets consisted of a unialgal diet of *Isochrysis galbana* (I) or a mixed species algal diet of *I. galbana* and *Rhodomonas reticulata* (I+R).

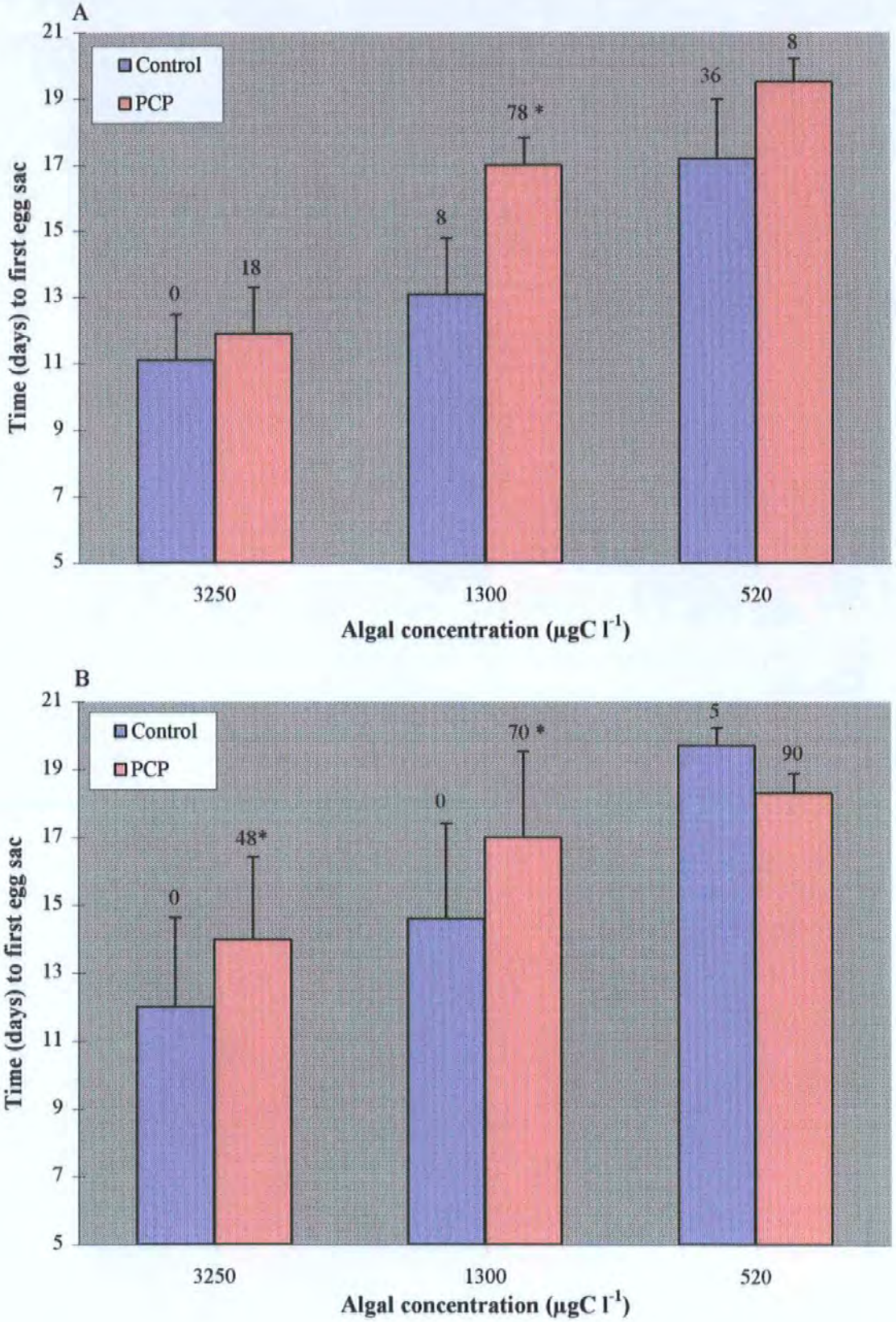


Figure 7.2. Time to first egg sac for surviving females in cohorts reared at 20°C and algal diets consisting of (A) *Isochrysis galbana* and *Rhodomonas reticulata* and (B) *Isochrysis* alone. Values represent mean ±1SD, numbers above each SD denote the % mortality of the copepods in each cohort (n=50) and * denotes a significant difference ($P < 0.05$) between control and PCP treatment.

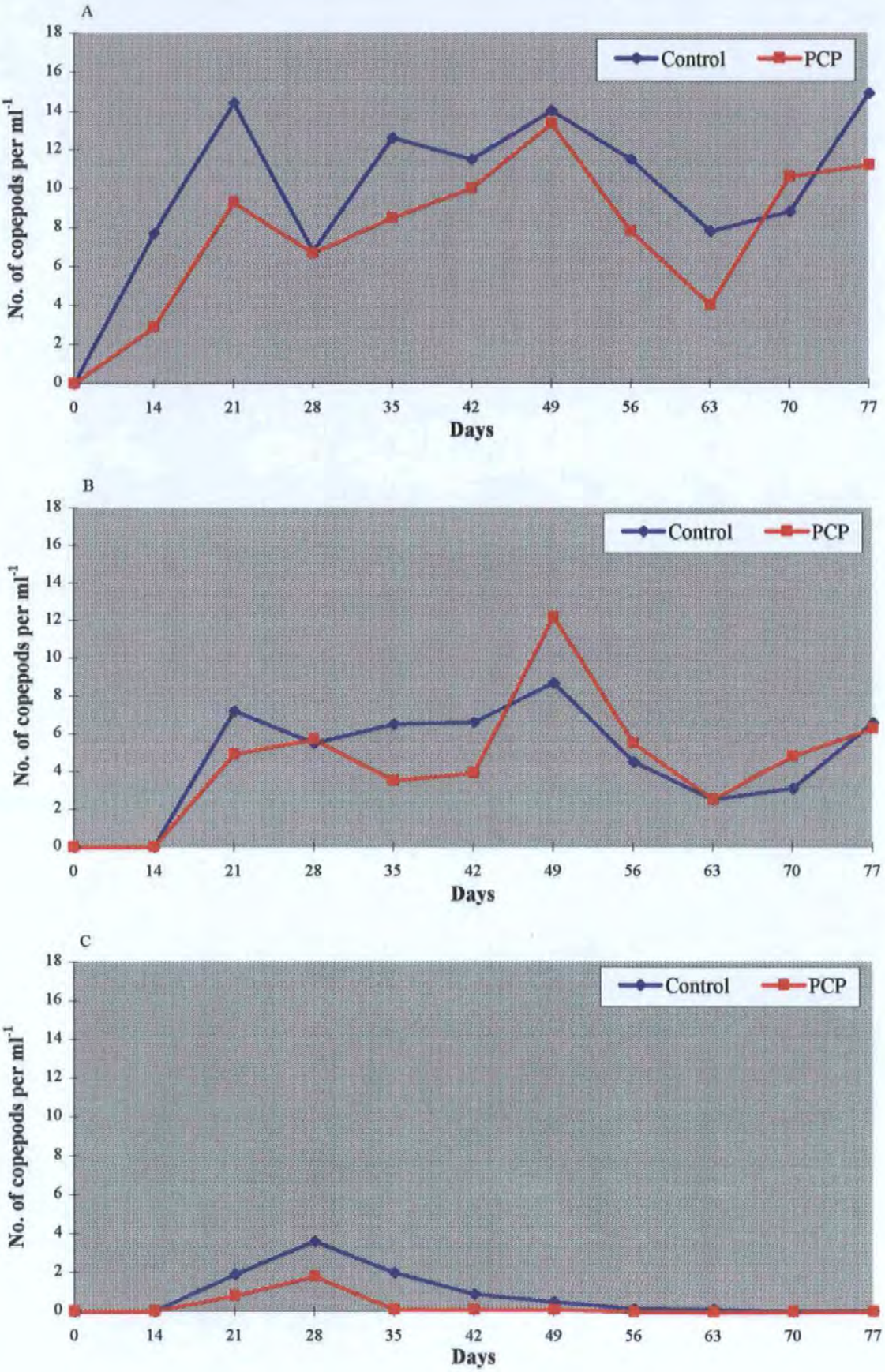


Figure 7.3. Comparison between population growth (mean number) of *Tisbe battagliai* exposed to control and 100 µg l PCP and fed on a mixed species algal diet consisting of (A) 3250, (B) 1300 and (C) 520 µgC l *Isochrysis galbana* and *Rhodomonas reticulata.* Variability surrounding the mean values is shown in Tables 7.3 - 7.5.

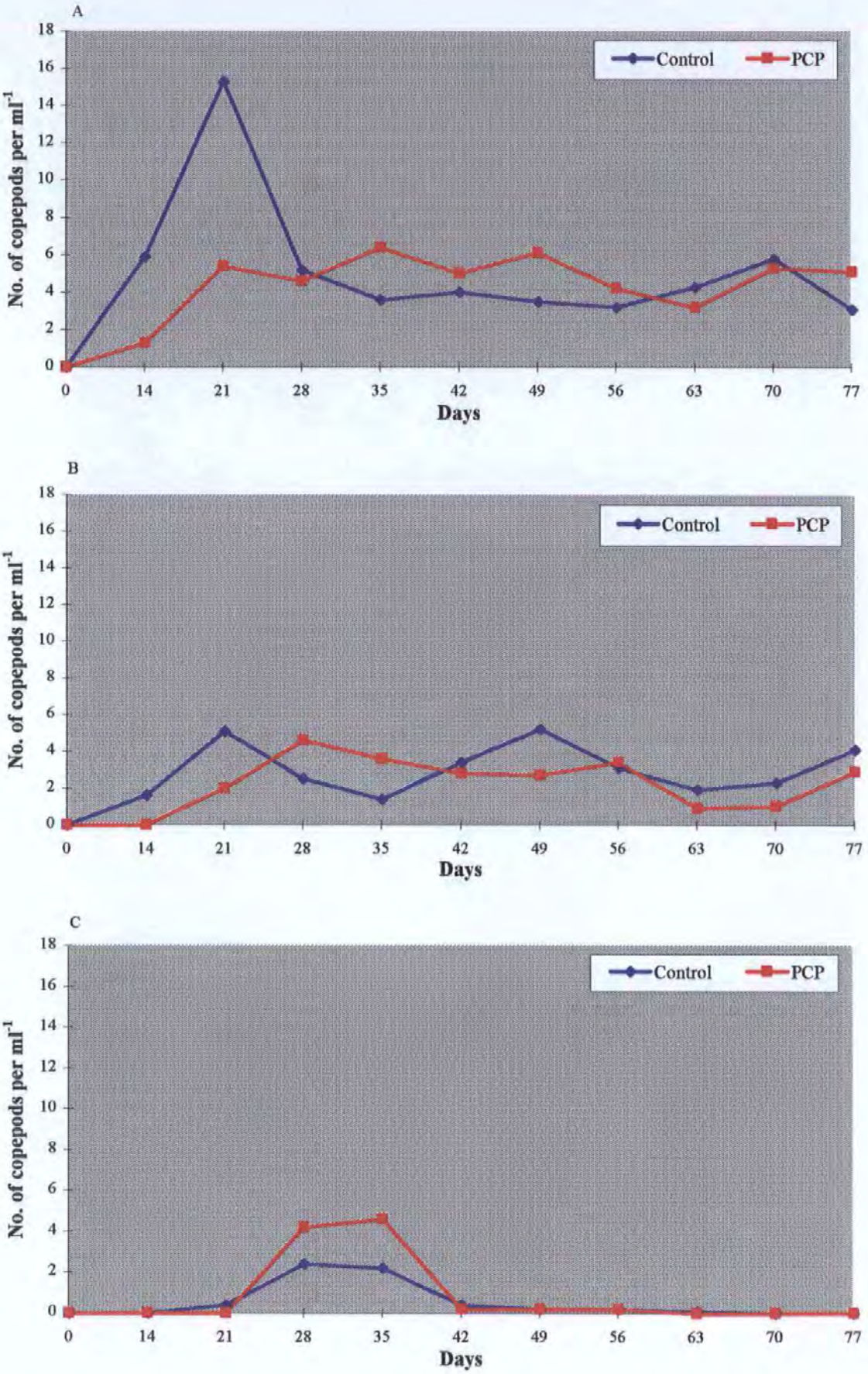


Figure 7.4. Comparison between population growth (mean numbers) of *Tisbe battagliai* exposed to control and 100 µg l PCP and fed on a unialgal diet consisting of (A) 3250, (B) 1300 and (C) 520 µgC 1 *Isochrysis galbana*. Variability surrounding the mean values is shown in Tables 7.3 - 7.5.

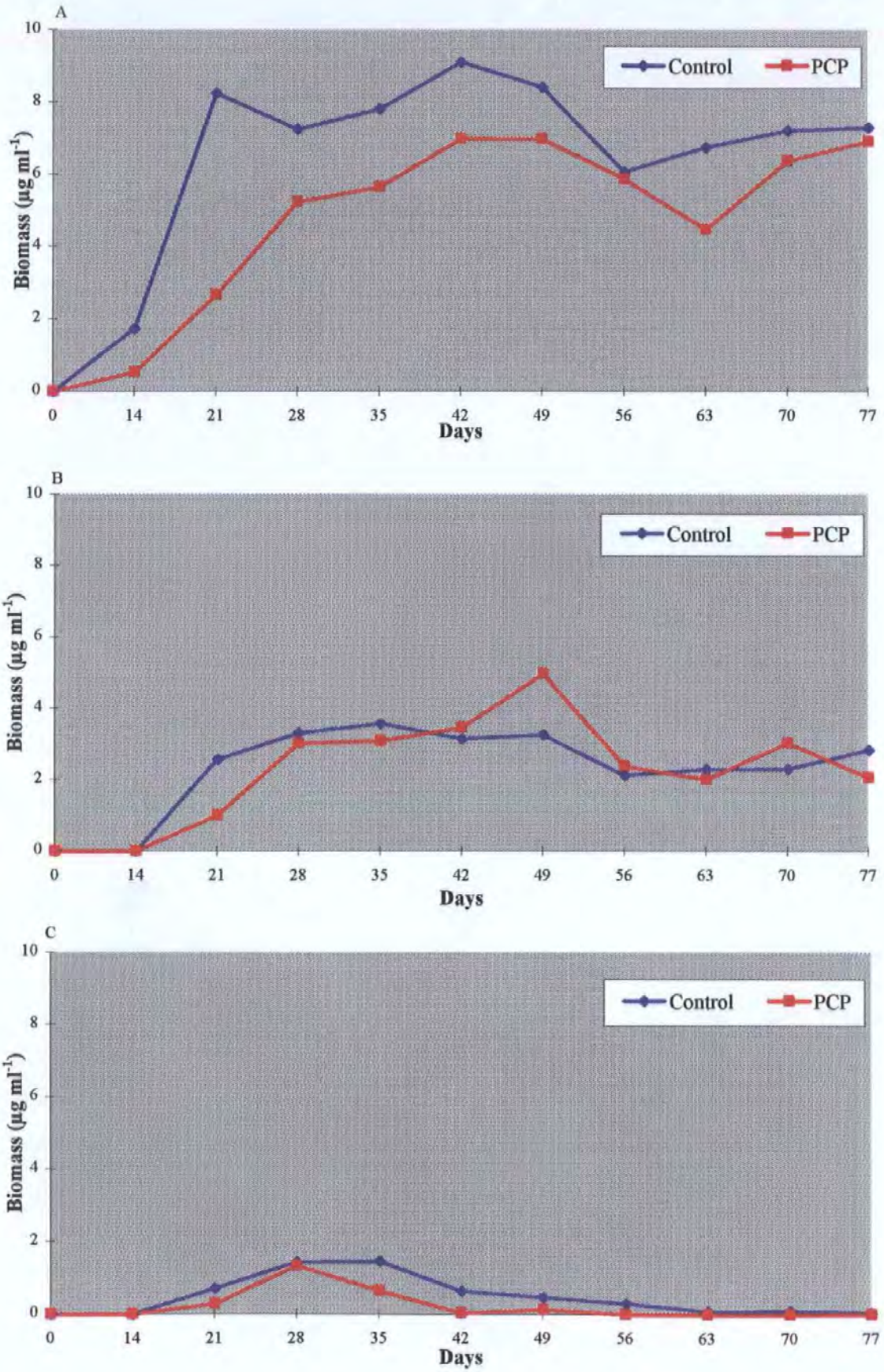


Figure 7.5. Comparison between population growth (mean biomass) of *Tisbe battagliai* exposed to control and 100 µg l PCP and fed on a mixed species algal diet consisting of (A) 3250, (B) 1300 and (C) 520 µgC l *Isochrysis galbana* and *Rhodomonas reticulata*. Variability surrounding the mean values is shown in Tables 7.3 - 7.5.

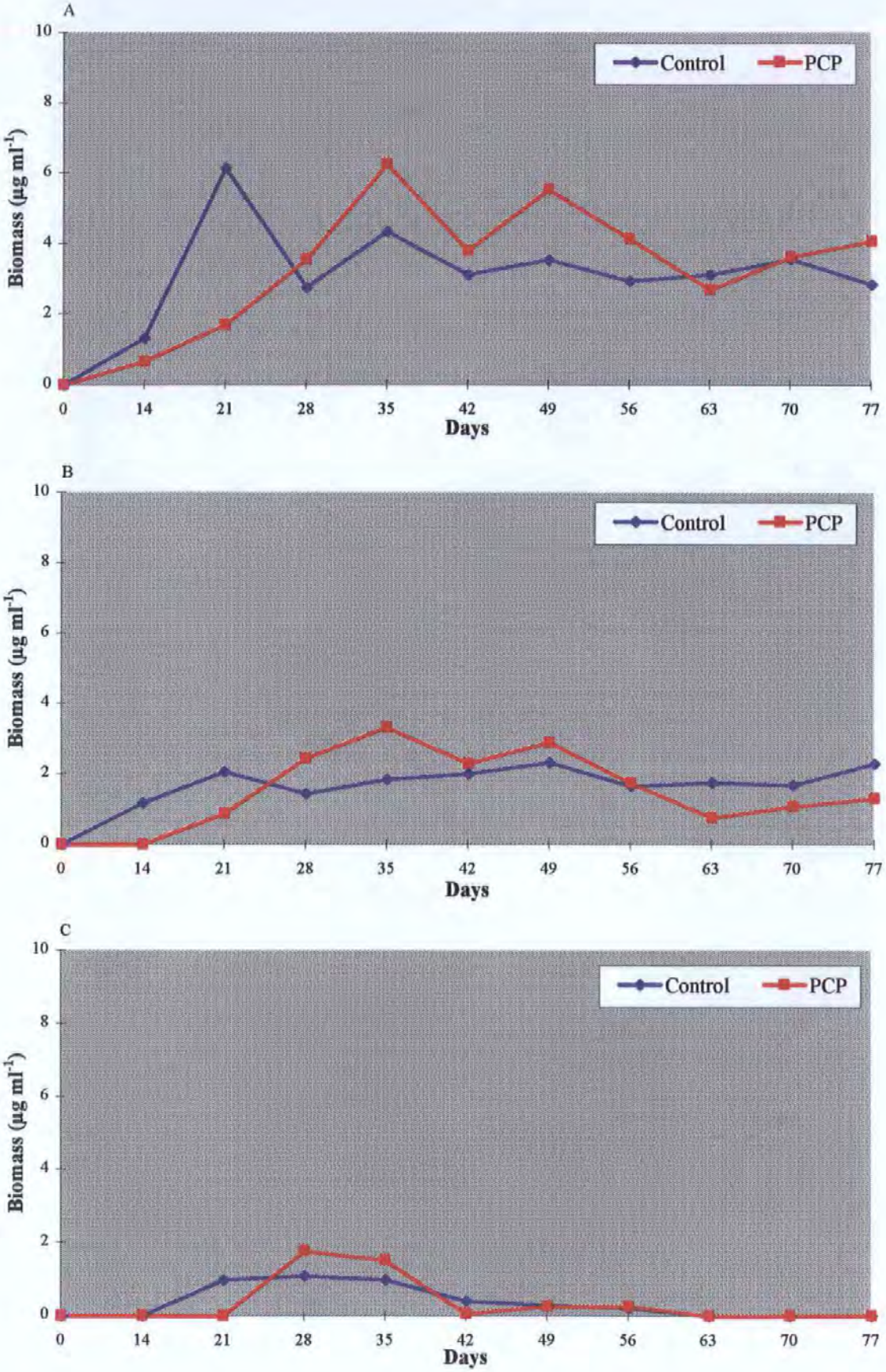


Figure 7.6. Comparison between population growth (mean biomass) of *Tisbe battagliai* exposed to control and 100 µg l PCP and fed on a unialgal diet consisting of (A) 3250, (B) 1300 and (C) 520 µgC l *Isochrysis galbana*. Variability surrounding the mean values is shown in Tables 7.3 - 7.5.

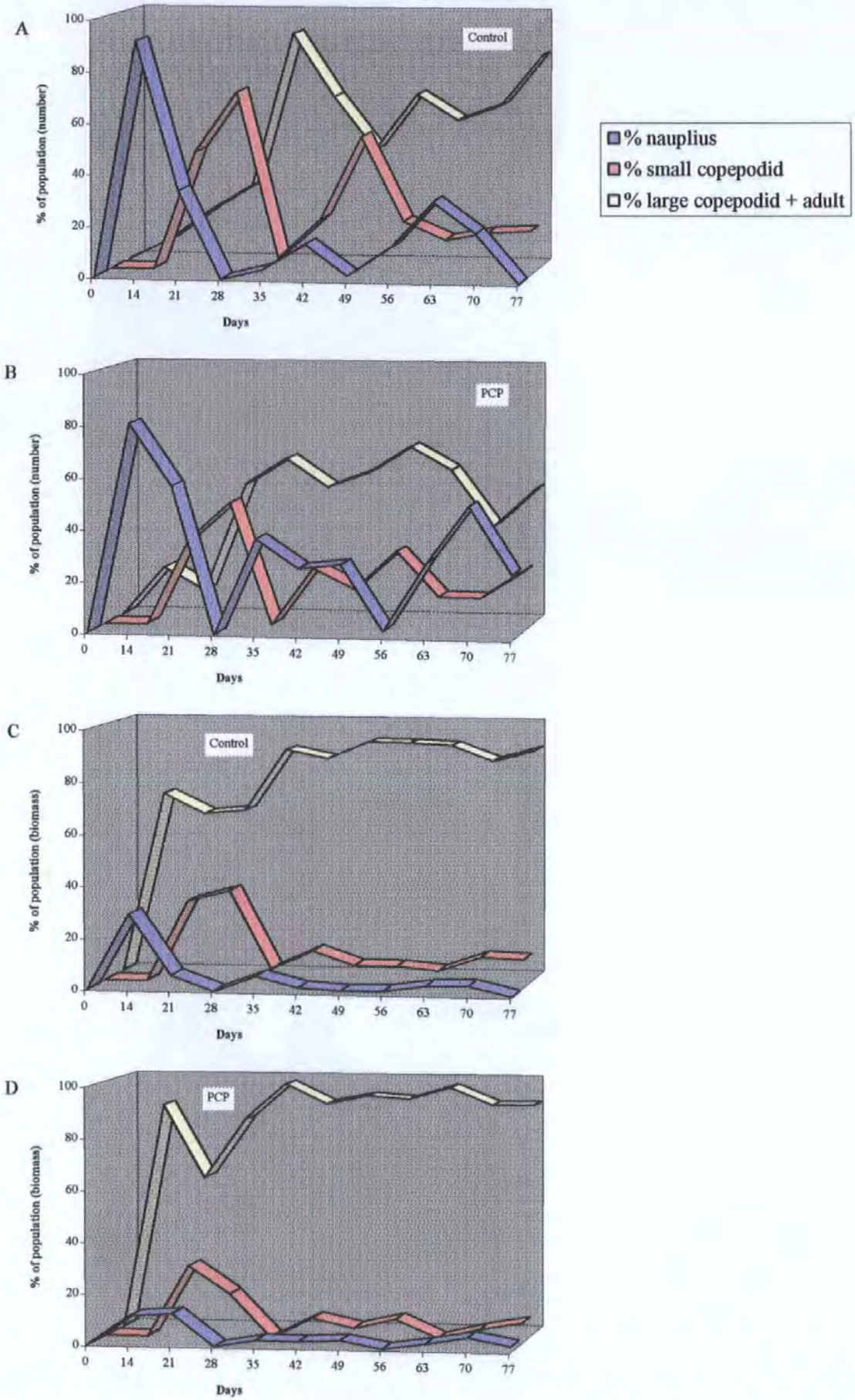


Figure 7.7. Population growth of *Tisbe battagliai* fed a unialgal diet of 3250 µgC l. Results represent the proportion of different life stages (mean values), as a % of the total population, for (A) numbers in the control and (B) numbers in the PCP treatment, (C) biomass in the control and (D) biomass in the PCP treatment.

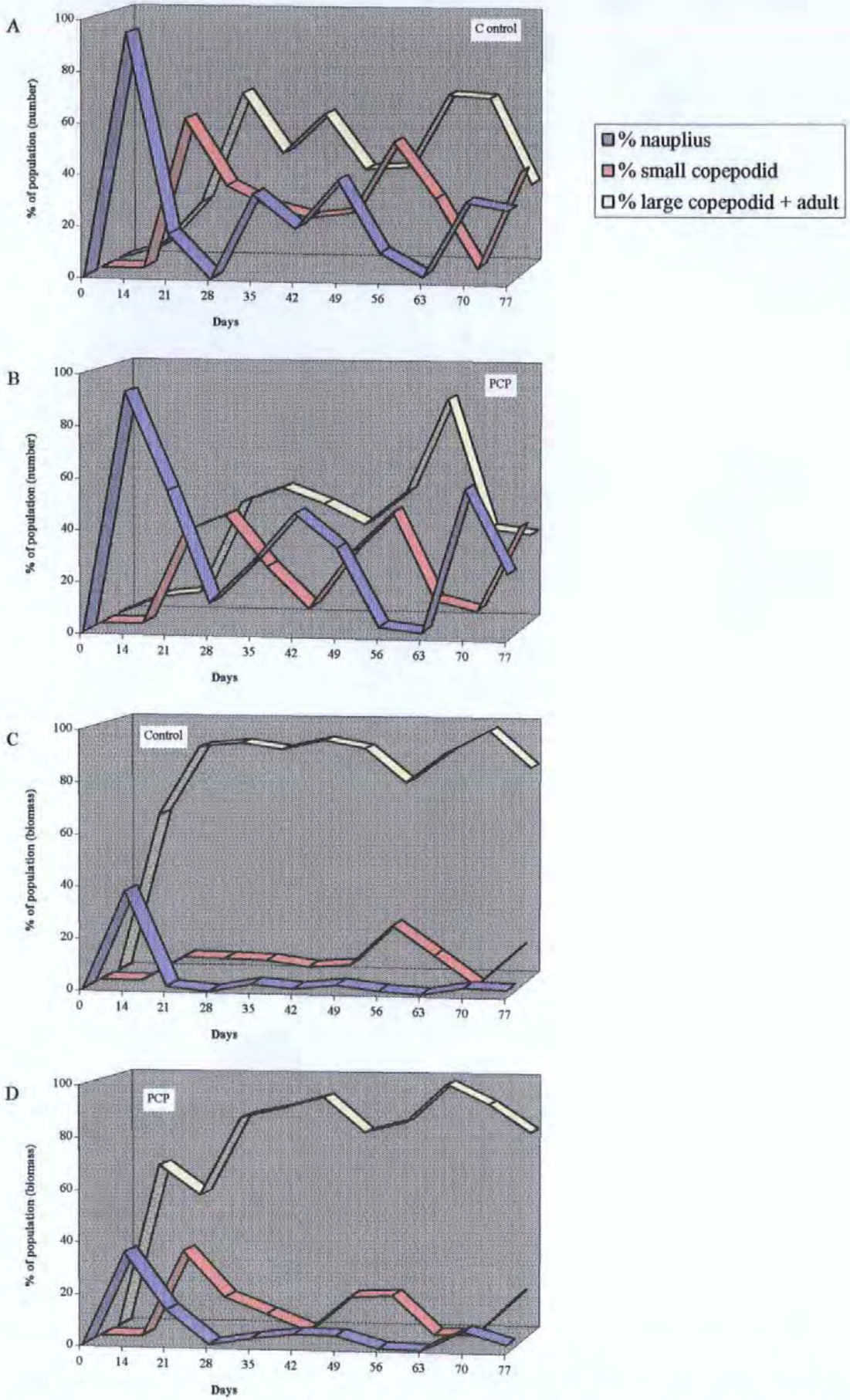


Figure 7.8. Population growth of *Tisbe battagliai* fed a mixed species algal diet of 3250 µgC l. Results represent the proportion of different life stages (mean values), as a % of the total population, for (A) numbers in the control and (B) numbers in the PCP treatment, (C) biomass in the control and (D) biomass in the PCP treatment.

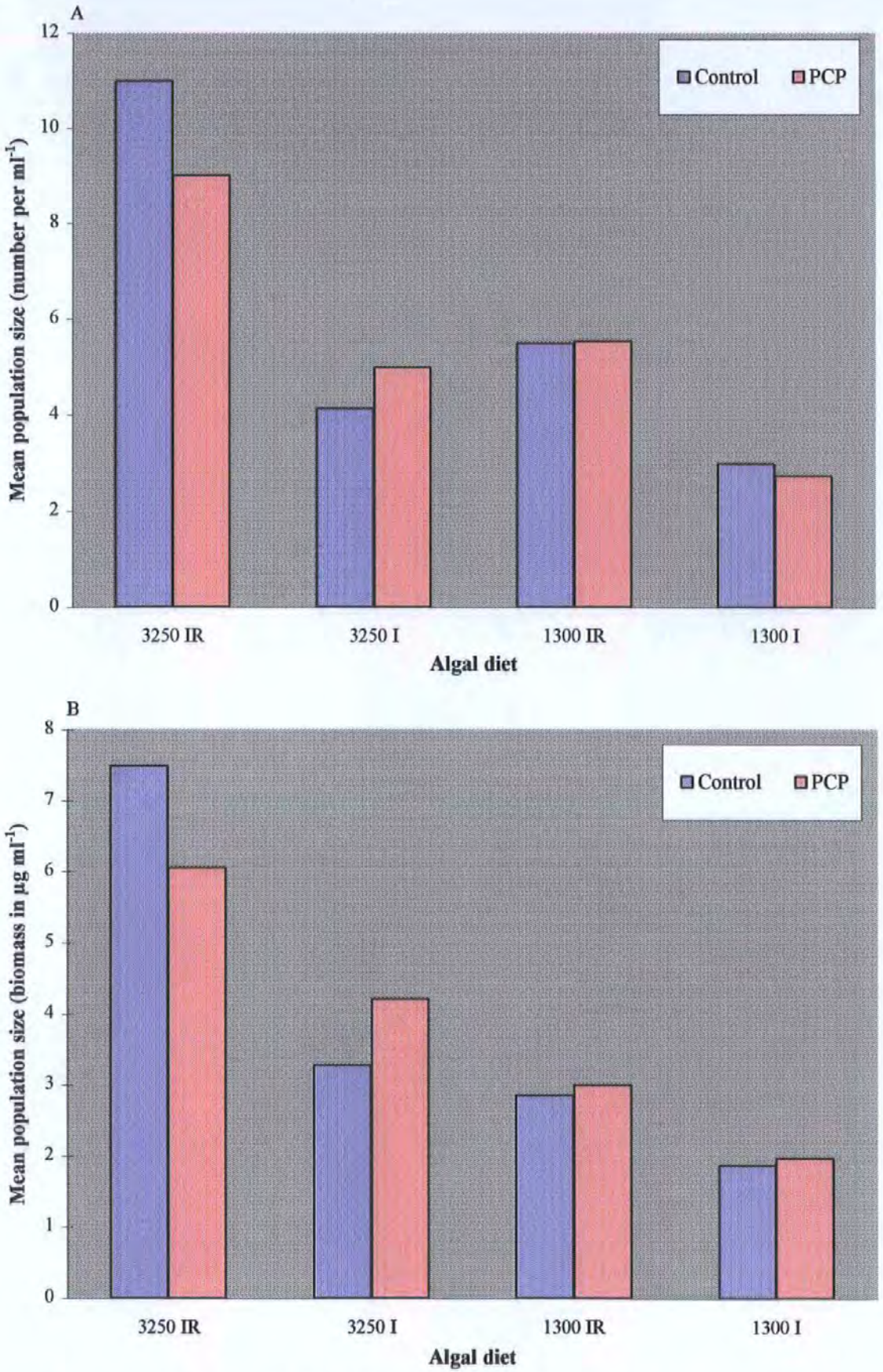


Figure 7.9. Changes in (A) number and (B) biomass for populations of *Tisbe battagliai* reared on 1300 or 3250 ugC l of a unialgal diet of *Isochrysis* (I) or a mixed species algal diet of *Isochrysis* + *Rhodomonas* (IR). Results represent mean values (n=8) for measurements on weeks 4 - 11, when each population had appeared to reach carrying capacity.

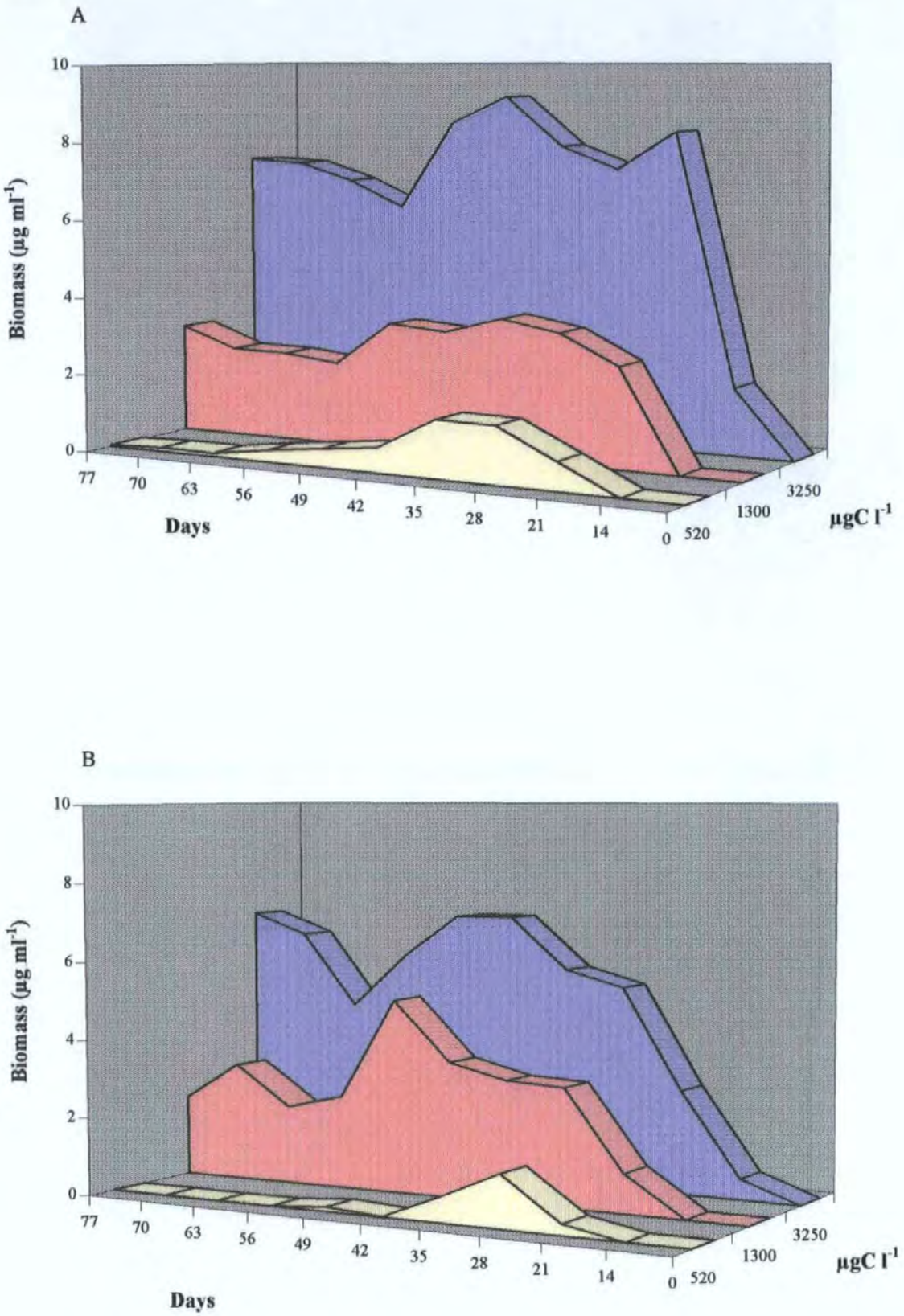


Figure 7.10. Increase in population size (mean biomass in µg dry weight per ml) of *Tisbe battagliai* reared at different algal concentrations (520, 1300 and 3250 ugC l) of a mixed species algal diet in (A) control and (B) PCP treatments.

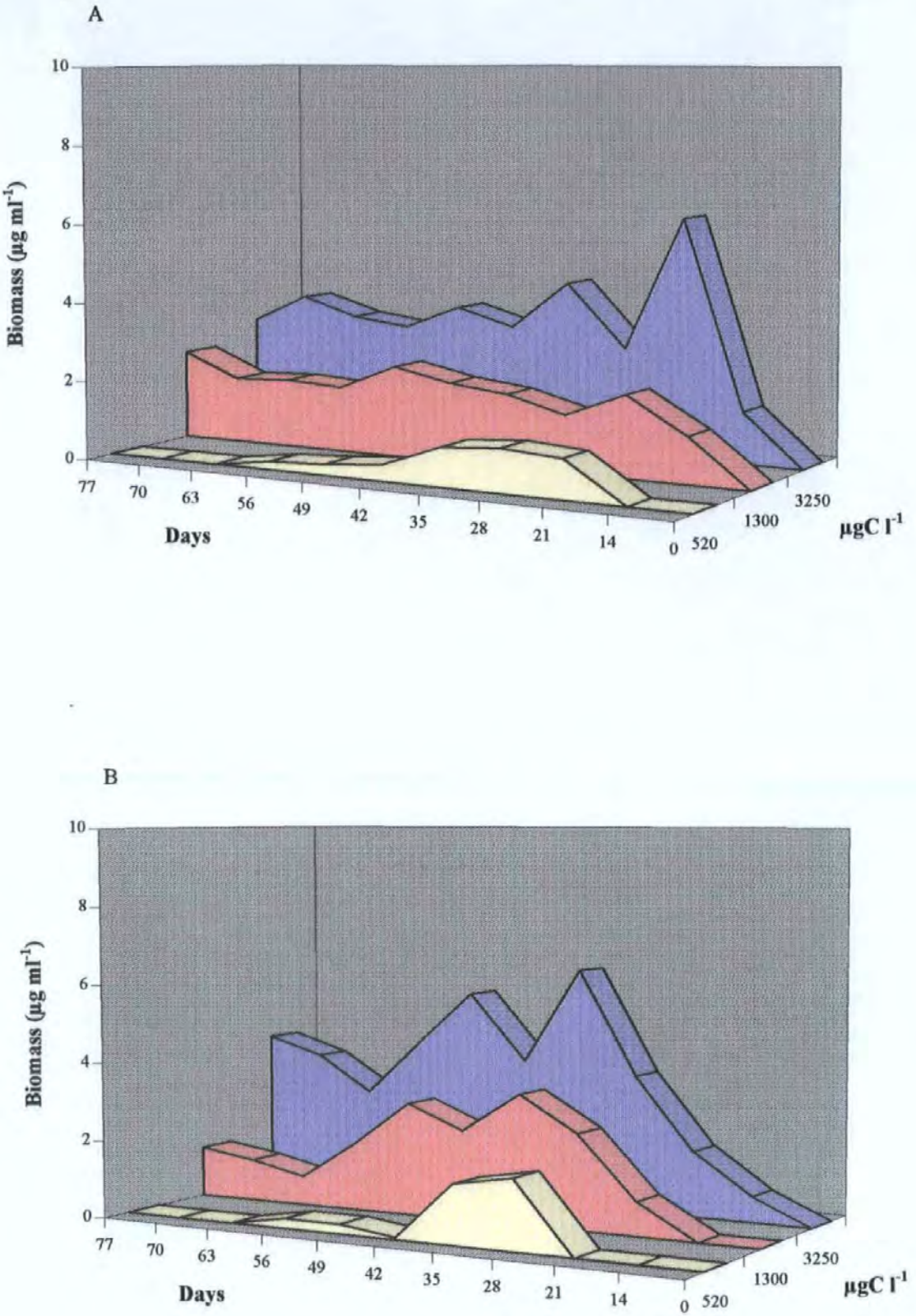


Figure 7.11. Increase in population size (mean biomass in µg dry weight per ml) of *Tisbe battagliai* reared at different algal concentrations (520, 1300 and 3250 ugC l) of a unialgal diet in (A) control and (B) PCP treatments.

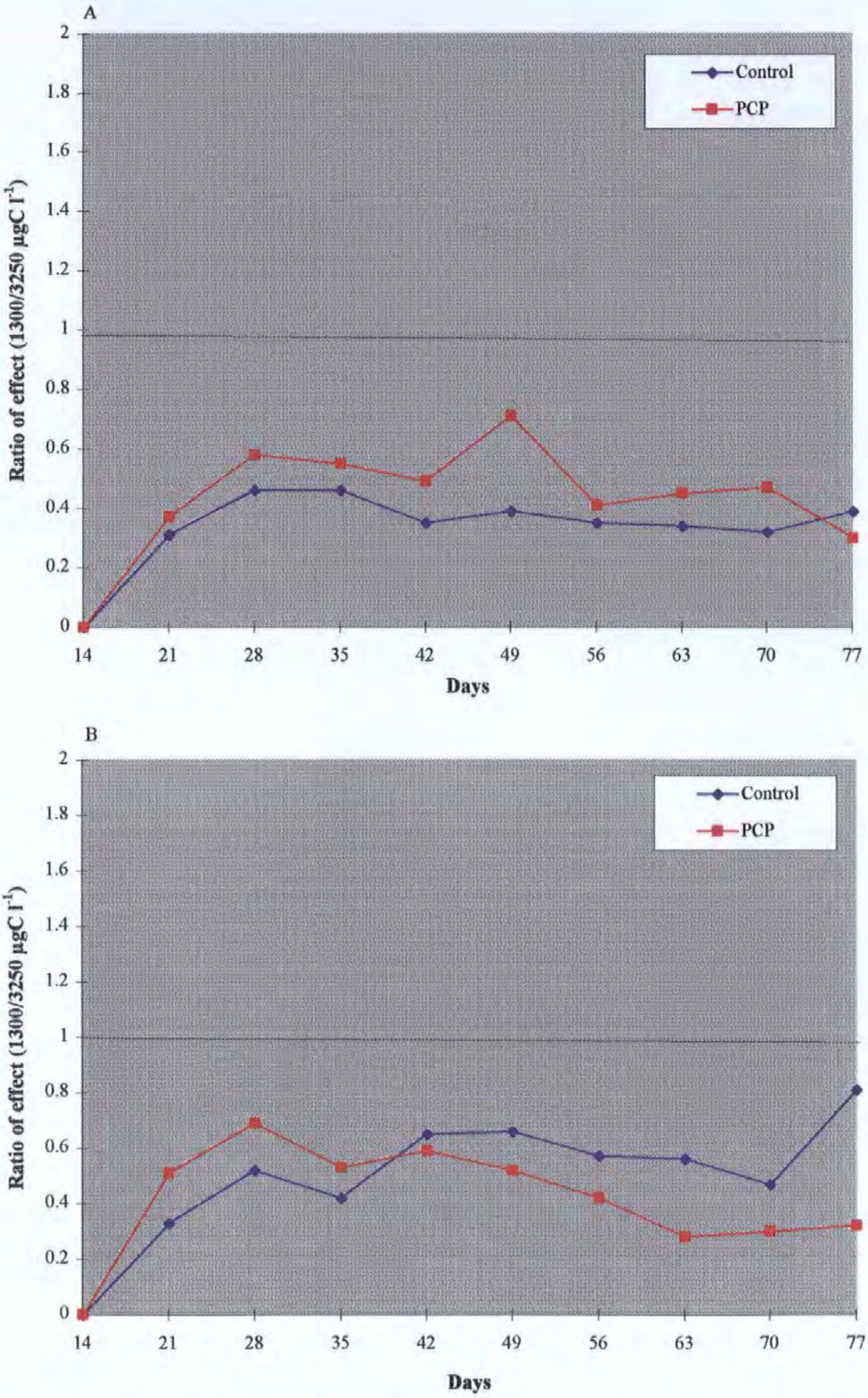


Figure 7.12. Changes in population size of *Tisbe battagliai* fed (A) a mixed species and (B) a unialgal diet. Results are expressed as a change in copepod biomass (µg dry weight) in control and PCP treatments between those fed 1300 and 3250 µgC l (ratio of mean values for 1300/3250 µgC l).

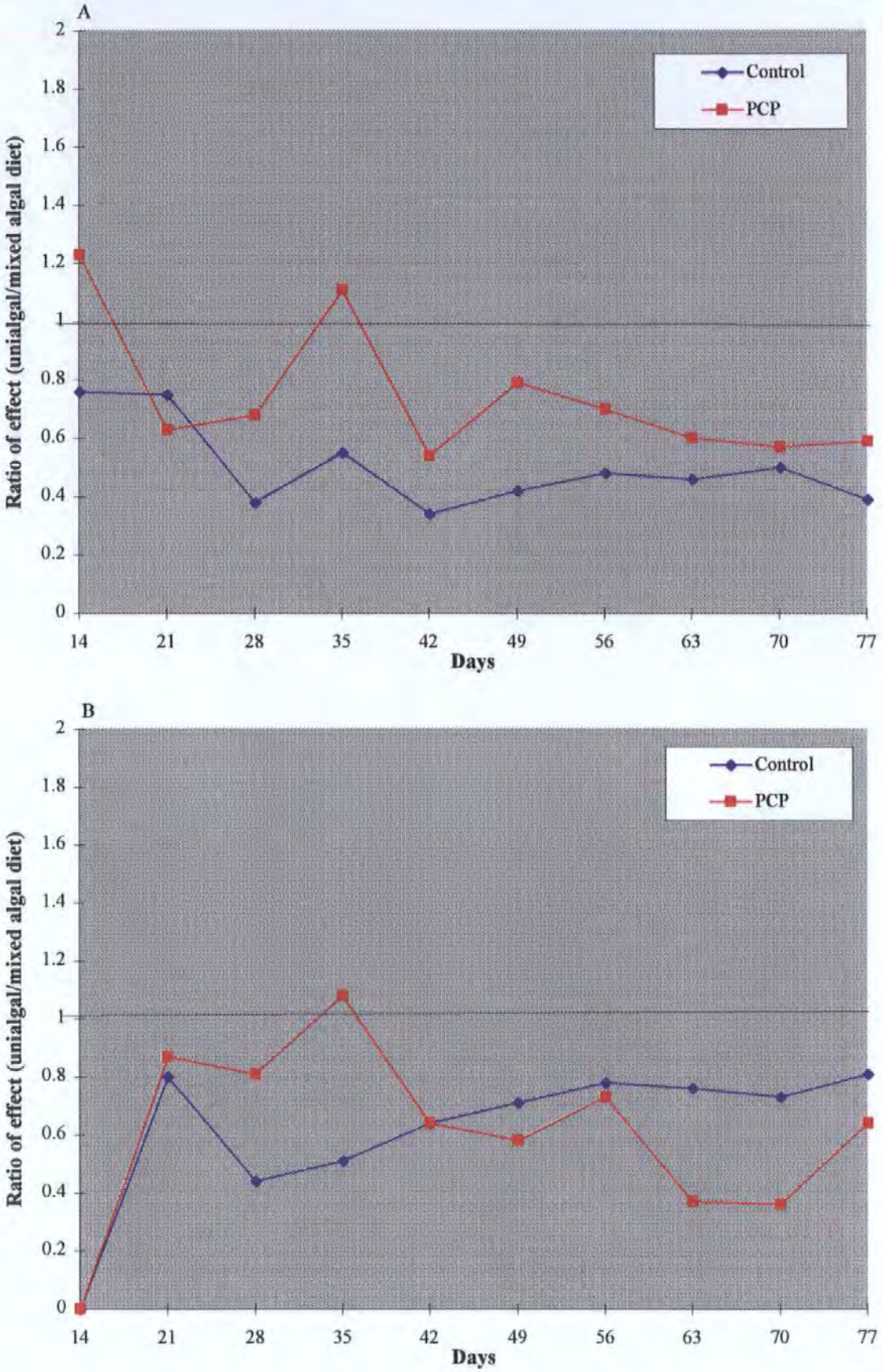


Figure 7.13. Changes in population size of *Tisbe battagliai* fed (A) 3250 µgC l and (B) 1300 µgC l of a unialgal or mixed species algal diet. Results are expressed as a change in copepod biomass (µg dry weight) between those fed corresponding concentrations of the unialgal and mixed algal diet (ratio of mean values for unialgal/mixed algal diet).

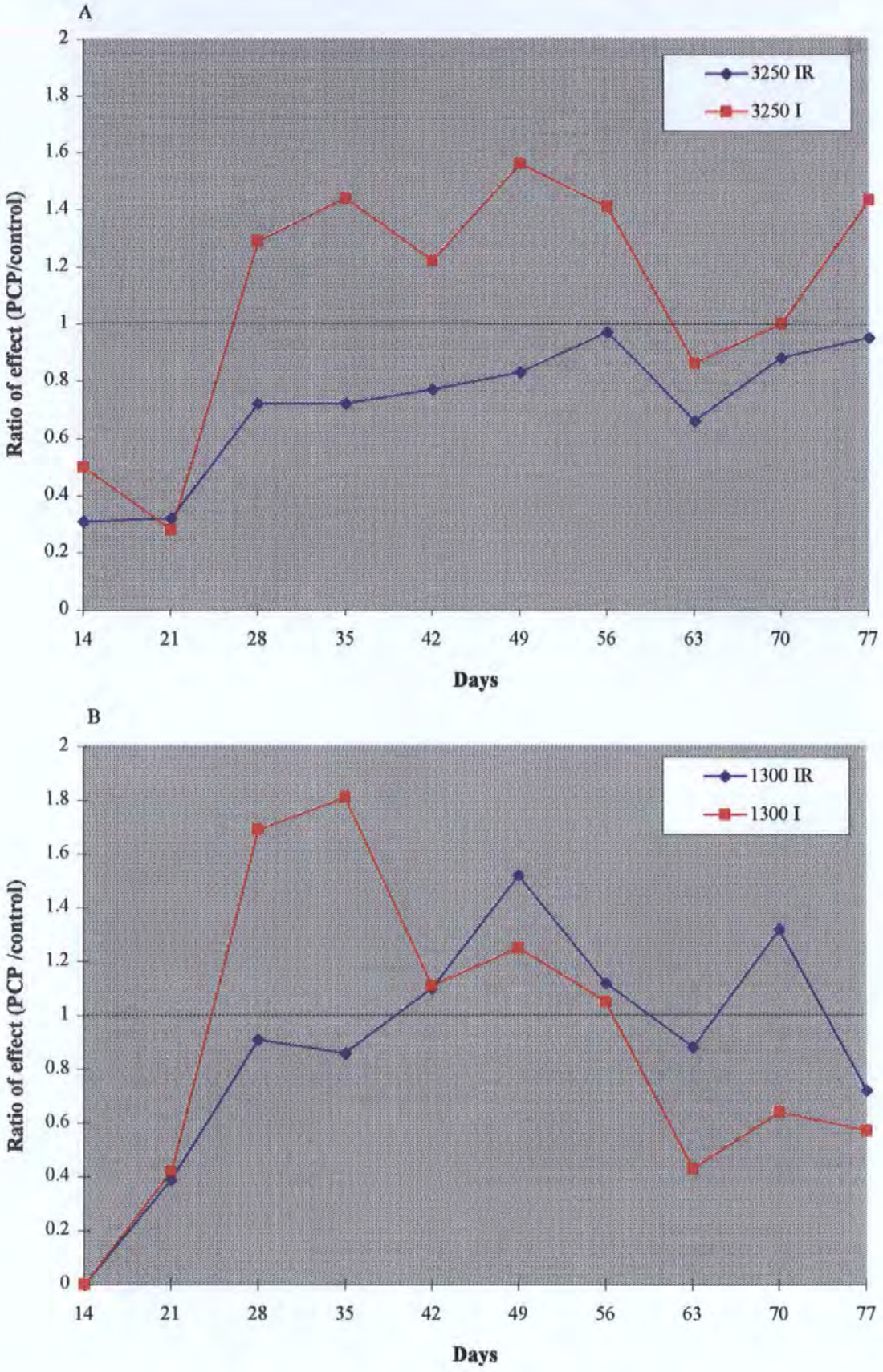


Figure 7.14. Changes in population size of *Tisbe battagliai* fed (A) 3250µgC l and (B) 1300 µgC l of a unialgal diet of *Isochrysis* (I) or a mixed species algal diet of *Isochrysis* and *Rhodomonas* (IR). Results are expressed as a change in copepod biomass (µg dry weight) between PCP and the corresponding control treatment (ratio of mean values for PCP/control).

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

A general discussion of the key findings from this research programme proceeds within the framework of the four research objectives set out at the start of this study (Section 1.7).

Objective 1: To develop chronic toxicity test methods using *Tisbe battagliai*

A suite of chronic toxicity test methods has been developed to measure the potential effect of chemical contaminants on postembryonic development (Chapter 2), reproduction (Chapter 3) and the complete life cycle (Chapter 4) of *T. battagliai*. The test methods were carefully chosen to meet a number of important criteria for the development of laboratory chronic toxicity test methods (Section 1.4). These criteria included the use of sensitive life stages, the use of realistic and relevant end points, establishing the links between contaminant effects at different levels of biological organisation, and the culture and availability of test organisms. The test procedures employed different biological end points and required different exposure periods, factors that influenced the time and effort required to complete these experiments. Although chronic toxicity tests require relatively long exposure periods, measurements of larval development and reproduction have been used to provide relatively short-term and cost-effective estimates of chronic toxicity (Section 1.6). These short-term measurements provide an estimate of potential chronic effects, however, to be of most value the end points should be validated with results from chronic exposures. The development of chronic test methods using *T. battagliai*, as described in this research programme, enables these validations to be made and provide a suite of techniques for measuring the potential long-term biological effects of contaminants on the marine environment.

The test procedures for postembryonic development and reproduction can be carried out separately or in combination. Combined measurements of development and reproduction take account of potential differences in sensitivity between the different life stages of the test organism, thereby, providing a more comprehensive assessment of the potential hazard posed

by the test chemical. With careful planning, the test procedures for development and reproduction can be carried out simultaneously. This approach provides a practical alternative to complete life-cycle exposures, which are more difficult and time consuming to perform. Practical considerations apply to each of the chronic toxicity test methods described in this research programme. Measurements of naupliar development to the adult stage require significant manipulation and observation of the copepods (Chapter 2). Also, because of sexual dimorphism and potential changes in sex ratio, the operator must use a relatively large number of copepods per treatment to ensure sufficient data for a robust statistical analysis of male and female development times. Measurements of reproduction, on the other hand, are relatively easy to perform, require fewer organisms, and the exposure duration can proceed for the duration of the female lifespan, or shorter, if required. Reproduction, however, is very sensitive to food quantity and quality and these factors will have a significant effect on test performance. Complete life-cycle exposures combine effects on development and reproduction within the same experiment and the fate of all individuals in a cohort is followed from birth to death. Relatively large numbers of copepods per treatment are required to account for variable sex ratios and, as a result, the size of the experimental design and subsequent experimental efforts are high. However, life-cycle exposures enable the construction of life tables and subsequent calculation of the population parameter r_m. This parameter has been proposed as a test parameter because it integrates age-specific survival and reproduction, thereby, providing a more ecologically relevant criterion than currently used toxicity test methods using separate measures of survival and reproduction. The chronic toxicity test procedures described above measure the fate of individual copepods, however, these procedures do not take into account population effects that occur through interactions between individuals, competition and food availability. Although the need to translate individual organism responses to higher levels of biological organisation has been identified (Section 1.4.4), few attempts have been made to

demonstrate a causal relationship between effects at different levels of biological organisation. The development of test methods for measuring the effect of chemicals on individuals and populations of *Tisbe battagliai* (Chapter 7) provides an experimental approach for comparing effects at different levels of biological organisation within the same species.

The chronic test methods described here may be applicable to other members of the *Tisbe* genus with little or no modification apart from choice of diet. Simple laboratory equipment was used for testing, space requirements were minimal and the techniques can be used for small sample volumes. The static culture renewal system was a simple procedure requiring little maintenance, and careful management of the cultures can provide a ready availability of copepods for testing throughout the year. The provision of algal food for the *Tisbe* diet is relatively time consuming, however, the operation of algal chemostats reduces the effort required. Present results have shown that food quantity and the nutritional quality of the algal food is a critical factor in the performance of *Tisbe* chronic tests, therefore, particular care and attention should be paid to the algal culture techniques. Although these harpacticoids are relatively small in size, particularly the naupliar stages, they can be handled and manipulated relatively easily after some practice. They are sufficiently robust to tolerate these procedures and it is most unusual to observe mortalities in the control treatments apart from natural phenomena when copepods are approaching the end of their lifespan. In the United Kingdom, there has been a gap in the availability of chronic toxicity test methods using indigenous species. The development of chronic methods using *Tisbe battagliai* offer considerable potential in this regard, however, further research is required to evaluate the performance of these test methods using a range of chemicals with different modes of action.

Objective 2: To establish information on the basic biology of *Tisbe battagliai*

The research programme has provided information on the influence of temperature, and food quantity and quality, on the population dynamics of *Tisbe battagliai* under laboratory conditions. These data provide a valuable insight into the potential importance of these environmental factors for influencing the development of populations of *T. battagliai* in the natural environment. These data also help to define the optimum conditions for culture and chronic toxicity testing of this species in the laboratory.

Temperature and food concentration had a significant effect on postembryonic development of *Tisbe battagliai* and development times decreased with increasing temperature and quantity of food (Chapters 2). There was a significant interaction between temperature and food concentration on reproduction and the optimal conditions for offspring production in the laboratory required high algal food concentrations (3250 $\mu gC\ l^{-1}$) and a temperature of 20°C (Chapter 3). Similar results were obtained from cohort life-table experiments (Chapter 4) and results confirmed that development and reproductive output in *Tisbe battagliai* is strongly temperature dependent. These results are consistent with published information on the effect of temperature on development and reproduction in marine harpacticoids (Hicks & Coull, 1983). By comparison, much less is known about the nutritional requirements of harpacticoids and how these animals respond to food resources by way of regulation of reproductive periodicity and population dynamics.

The effect of food quality on the population dynamics of *Tisbe battagliai* was investigated using mixed species and unialgal diets (Chapter 5). The results from these experiments suggest that food quality is an important determinant of population dynamics and that specific components of the copepod diet may be essential for reproduction. In the field situation, the availability of food resources, and their nutritional value for copepods, could be the major factor affecting the reproductive activity of phytal harpacticoids (Hicks, 1977).

Results showed that *Rhodomonas reticulata*, alone or in combination with *Isochrysis galbana*, was a superior diet for *Tisbe battagliai* than *I. galbana* alone. Consequently, the choice of what algal species to use in the copepod diet is important and a mixed algal diet (dietary diversity) potentially increases the likelihood that a nutritionally complete ration will be obtained. If dietary diversity is the clue to providing a nutritionally optimal diet, those species that are able to utilise a wide range of different foods are likely to have a competitive advantage over those species regarded as dietary specialists. The importance of food quantity and quality for the population dynamics of *Tisbe battagliai*, as determined by measurement of individual copepods (Chapters 2 to 4), were confirmed by population experiments (Chapter 7). Data from population experiments present evidence that intraspecific competition tends to regulate population size within relatively narrow limits. After populations had reached steady state (or carrying capacity), the final population density of *T. battagliai* was influenced by food quantity and quality.

The results from this research programme suggest that temperature and food availability (quantity and quality) will have strong influences on the reproductive parameters of *Tisbe battagliai* in field populations, however, the extrapolation from laboratory results to the field situation must be undertaken with caution. The factors controlling species composition and abundance of harpacticoids in the phytal biotope are complex, and the precise way in which temperature and/or food supply conspire to influence in situ reproductive periodicity with (via recruitment) its attendant effects on species abundance remains to be fully and critically evaluated (Hicks, 1985).

Objective 3: To improve the environmental applicability of laboratory toxicity tests

Established laboratory toxicity test procedures do not take account of the degree of complexity encountered in the natural environment and this underlines the difficulties of

extrapolating from the laboratory to the field situation. The results from Chapter 6 indicate that the response to toxicants depends on environmental conditions (e.g. food concentration and associated nutritional quality) and failure to consider such factors in laboratory toxicity tests may increase the uncertainty for correctly predicting the effects of chemicals under the range of environmental conditions that occur in the natural environment. It may be possible to improve the ability of laboratory tests to predict those in the field by performing such tests under a range of environmental conditions.

The sensitivity of *Tisbe battagliai* to PCP was influenced by environmental factors (e.g. food concentration and diet type) and such factors are usually ignored in standard laboratory toxicity tests. Meyer *et al.* (1987) cautioned that a single statistic such as r_m is not sufficiently robust to describe fully the population dynamics over a wide range of population densities and food availabilities. In the laboratory, standard chronic toxicity tests are performed at low animal densities (usually individuals) and food is supplied in excess. However, under suboptimal conditions (e.g. food limitation), density-dependent competition for food could become important and population growth rates could decline. With these caveats, Meyer *et al.* (1987) concluded that r_m can be extremely useful for evaluating apparently conflicting effects of pollutants on survival and reproduction, and for determining the population-level significance of organism-level responses to pollutants. Similar conclusions were drawn by Gentile *et al.* (1982, 1983) who suggested that the effects of other factors (e.g. predation) should be considered in combination with toxicant stress to predict population growth rates. Results with *Tisbe battagliai* show that life-table data were dependent upon environmental factors such as temperature, food concentration and diet composition (Chapters 4 and 5), and upon interactions between these key environmental variables and the test chemical (Chapter 6). Consequently, the use of life tables to measure the potential risk posed by long-term exposure to chemicals should take these (environmental) factors into consideration. For example, life-

table analysis can be used to define the environmental variables (e.g. food concentration) that are most important, or critical, for population growth of a particular species. Measurements of toxicity, conducted at critical, instead of optimal environmental conditions, could help to define the biological effects of chemicals under worst case, or more environmentally realistic, exposure conditions.

The investigation of potential interactions between key environmental factors and toxicants on the biological response of the test organism was an important objective for this research programme. This objective was difficult to achieve and required significant experimental effort compared with traditional toxicity test approaches (in which environmental variables are predetermined) for measuring the potential chronic toxicity of chemicals. In the field, interactions between environmental variables, chemical contaminants and the biological response of organisms are most likely to occur at sublethal exposure concentrations. In the case of PCP, there was a relatively narrow response threshold between acute and chronic toxicity, therefore, it was difficult to define exposure concentrations that captured sublethal effects on development and reproduction without inducing significant mortality. Despite these difficulties, it was possible to identify significant interactions between the chosen environmental variables (e.g. temperature), PCP and subsequent biological effects on *Tisbe battagliai* (Chapter 6). Further experiments are required to identify whether such interactions, and their subsequent effects on the toxicity of chemicals to organisms, are the rule rather than the exception.

Objective 4: To develop test procedures for linking effects at different levels of biological organisation

A key feature of population experiments, and a major difference from established chronic toxicity test procedures, is that effects of the chemical on all stages of the life cycle are

considered. For organisms with rapid generation times, population experiments of sufficient duration may include exposure to more than one generation of organisms hence measurements of survival, development, reproduction and offspring fitness are included. Furthermore, long-term, multigeneration exposure of organisms to sublethal concentrations of chemicals at the population level may involve processes of physiological acclimation and genetic adaptation. These factors are not considered in established chronic toxicity test procedures yet they may provide a more valid interpretation of the potential long-term effects of chemicals on populations of organisms in the field. The use of a combined approach to measure (and predict) the potential effects of chemicals at different levels of biological organisation (individual and population level) should provide a greater understanding of the ecological relevance of the results from toxicity tests.

The development of chronic test methods for *Tisbe battagliai* makes it possible to investigate the predictive links between effects at the individual and population level. However, there are limitations associated with laboratory population experiments and the extrapolation of results to the field situation. Laboratory population experiments are an over simplification of events in the field, where individuals are affected not only by intraspecific competition but also by predators, competition from other species and changes in environmental factors. Several authors have investigated how predation by higher trophic levels may affect the structure of benthic harpacticoid populations. Hoppenheit (1975ab, 1976) performed a series of experiments to investigate the potential effects of predation on the population size of *Tisbe holothuriae*. Populations were exploited (a proportion removed) to represent predation and results from an observation period of 24 weeks (20 generations) indicated significant alterations of the population from long-term, constant predation pressure. More recently, Woods and Coull (1992) measured changes in population size of the benthic harpacticoid *Amphiascus tenuiremis* using 90% and 50% replacement of the population to

mimic the effects of predation. High reproductive potential, measured by a 28 fold increase in population size over 21 days (one generation), suggested that the effects of predation would not be enduring. Woods and Coull (1992) suggested that most harpacticoid species have the ability to out-reproduce any removal due to predation provided that predation pressure is not continuous. Predators of phytal harpacticoids have not been defined adequately (Hicks, 1980) but they may be expected to have an important influence on populations dynamics in the field if constant predation pressure is applied. It is worth noting that the ovigerous female is the life stage potentially most visible to predators and their size selective removal by predators may have important consequences for population structure.

There has, and continues to be, considerable debate concerning the relevance of single-species laboratory tests for predicting the effect of pollutants on ecosystems (Forbes & Forbes, 1994; Grothe *et al.*, 1996). Interactions amongst species, and between abiotic and biotic components of ecosystems, are the primary factors preventing accurate predictions from single species to higher levels of biological organisation (Forbes & Forbes, 1994). In the field, aquatic communities are complex and too poorly understood to study them directly, and cost-effectively, to determine whether toxicity is causing impairment (Mount, 1988). In the marine environment, most receiving water samples do not show acute toxicity, therefore, methods are required to measure the potential long-term (i.e. chronic) effects of contaminants. Against this background, regulatory approaches to pollution prevention require actions to be taken to prevent the release of contaminants before environmental alterations occur. Under these circumstances, laboratory tests will remain an important and cost-effective tool for the control and monitoring of contaminants

In conclusion, the test methods using *Tisbe battagliai* provide a suite of techniques that can be used to measure the chronic toxicity of single chemicals and aqueous effluents. The

methods are relatively simple to perform, are amenable to standardisation and provide relatively cost-effective measurements of chronic toxicity. These are important attributes for test methods used in regulatory ecotoxicology and the reproduction method is currently being evaluated by the Environment Agency for measuring the potential chronic toxicity of aqueous effluents (*pers. comm.*). The test methods can be used to provide chronic toxicity data but, more importantly, they can be used to address some of the current limitations associated with single-species laboratory tests. For example, used in conjunction with key environmental variables, the methods provide a greater understanding of the potential interaction between contaminants and abiotic variables, thereby, improving the extrapolation of laboratory results to the field situation. The ability to carry out measurements on individual and populations of *Tisbe battagliai* provides a valuable insight into the predictive links between effects at different levels of biological organisation.

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