IMPACTS OF MULTISTRESSORS ON THE SURVIVAL AND LIFE HISTORY TRAITS OF *Daphnia pulex*

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

GİZEM BEZİRCİ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

SEPTEMPER 2008

Approval of the thesis:

IMPACTS OF MULTISTRESSORS ON THE SURVIVAL AND LIFE HISTORY TRAITS OF *Daphnia pulex*

submitted by **GİZEM BEZİRCİ** in partial fulfillment of the requirements for the degree of **Master of Science in Biology Department**, **Middle East Technical University** by,

Prof. Dr. Canan Özgen
Dean, **Graduate School of Natural and Applied Sciences** _____

Prof. Dr. Zeki Kaya
Head of Department, **Biology** _____

Prof. Dr. Meryem Beklioğlu Yerli
Supervisor, **Biology** _____

Examining Committee Members:

Prof. Dr. Feride Severcan
Biology Dept., METU _____

Prof. Dr. Meryem Beklioğlu Yerli
Biology Dept., METU _____

Assoc. Prof. Dr. Sertaç Önde
Biology Dept., METU _____

Asst. Prof. Dr. Feriha Yıldırım
Graduate School of Natural and Applied Sciences,
Gazi University _____

Asst. Prof. Dr. Tahir Atıcı
Biology Dept., Gazi University _____

Date:05.09.2008

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name: GİZEM BEZİRCİ

Signature :

ABSTRACT

IMPACTS OF MULTISTRESSORS ON THE SURVIVAL AND LIFE HISTORY TRAITS OF *Daphnia pulex*

Bezirci, Gizem

M.Sc., Department of Biology

Supervisor: Prof. Dr. Meryem Beklioğlu Yerli

September 2008, 107 pages

As *Daphnia* have an important role in freshwater food webs, it is important to understand how environmental stressors affect their survival and life history traits.

Daphnia pulex were first acutely exposed to a combination of NaCl salinities (0.00-10.0 g/L) and fish-exuded kairomone. The 24 and 48 hour LC50 values were 0.401 and 0.159 g/L in kairomone-absence and 1.962 and 1.007 g/L in kairomone-presence. Hence, survival decreased with increasing salinity, while the kairomone enhanced daphnid resistance to salinity below 2 g/L.

During the chronic exposure to salinity (0.00-1.5 g/L) combined with the fish-exuded kairomone, impacts of temperature and food were also

investigated. Survival decreased significantly with increased salinity, while the kairomone had a positive impact on survival at intermediate salinity levels, suggesting an antagonistic relationship. Temperature alone had a significant negative impact on survival and its combined effect with salinity and fish kairomone was synergistic. However, the impact of food limitation was insignificant.

Life history parameters were affected by both salinity and the fish-exuded kairomone, the combination of which significantly decreased the body length: width ratio. Egg number/individual decreased with salinity and increased in the presence of the fish kairomone.

In conclusion, the combined impact of salinity and fish-exuded kairomone significantly affected daphnid survival and life history traits in a non-linear manner, such that fish kairomone enhanced daphnid resistance to intermediate salinity levels. Moreover, the combined effect of salinity, temperature and fish kairomone on daphnid survival was also significant.

Keywords: Toxicology, *Daphnia*, multistressors

ÖZ

ÇOKLU STRES FAKTÖRLERİNİN *Daphnia pulex*'İN HAYATTA KALMA ORANI VE YAŞAMA DÖNGÜSÜ ÜZERİNDEKİ ETKİLERİ

Bezirci, Gizem
Yüksek Lisans, Biyoloji Bölümü
Tez Yöneticisi: Prof. Dr. Meryem Beklioğlu Yerli

Eylül 2008, 107 sayfa

Daphnia tatlı su besin zincirinde önemli bir role sahip olduğundan, çevresel stres faktörlerinin yaşama oranı ve yaşam döngüsü parametreleri üzerindeki etkilerini bilmek önem taşımaktadır.

Daphnia pulex, öncelikle 0.00-10.g/L NaCl tuzluluk dozları ve balık sinyali kombinasyonuna akut olarak maruz bırakılmıştır. 24 ve 48 saatlik LD_{50} değerleri, balık sinyali yokluğunda 0.401 g/L ve 0.159 g/L, balık sinyali varlığında 1.962 g/L ve 1.007 g/L olarak tespit edilmiştir. Artan tuzlulukla yaşama oranı azalırken, 2 g/L tuzluluğun altında, balık sinyalinin varlığı, daphnia bireylerinin tuzluluğa olan direncini arttırmıştır.

Sıcaklık, tuzluluk ve balık sinyali kombinasyonunun daphnia üzerinde sinerjik etkisi olması ve yaşama oranını anlamlı bir şekilde azaltması yanında artan sıcaklık tek başına yaşama oranını olumsuz etkilenmiştir. Besin sınırlanmasının etkisi ise görülmemiştir.

Tuzluluk (0.00-1.5 g/L) ve balık sinyali kombinasyonunun kronik etkisinin yanında, sıcaklık ve besin etkiside araştırılmıştır. Artan tuzlulukla yaşama

oranını anlamlı azalırken, ara dozlarda balık sinyali varlığı, daphnia bireylerinin yaşama oranını pozitif etkilemiş ve bu ilişkinin antagonist olduğu önerilmiştir. Sıcaklık tek başına yaşama oranını negatif etkilemiştir, tuzluluk ve balık sinyali ile kombinasyonu ise sinerjiktir. Fakat besin sınırlanmasının anlamlı etkisi görülememiştir.

Yaşam döngüsü parametreleri, hem tuzluluk hemde balık sinyali varlığından etkilenmiştir ve bunların kombinasyonu vücut uzunluğu/vücut genişliği oranını anlamlı şekilde azaltmıştır. Yumurta sayısı/birey oranı tuzluluk ile azalmış, balık sinyalinin varlığı ile artımıştır.

Sonuç olarak, tuzluluk ve balık sinyali kombinasyonu, *Daphnia* hayatta kalma oranını ve yaşam döngüsü parametrelerini anlamlı doğrusal olmayan bir şekilde etkileşmiş, ara tuzluluk dozlarında balık sinyalinin varlığı daphnia bireylerinin dayanıklılığını arttırmıştır. Dahası, tuzluluk, sıcaklık ve balık sinyali kombinasyonu, *Daphnia pulex*'in hayatta kalma oranını anlamlı olarak etkilemiştir.

Anahtar Kelimeler: Toksikoloji, *Daphnia*, çoklu stres etmenleri

To my beloved family and friends

ACKNOWLEDGEMENTS

First, I would like to express my sincere thanks to my advisor Prof. Dr. Meryem Beklioğlu Yerli for her constant encouragement and support throughout my study. Without her ambition, creative ideas, clear expression and concern of the detail, this thesis would not have been possible. I am also thankful to Ali Sargun Tont, for his precious advices and all-time motivation.

I am also grateful to members of Limnology Lab; Ayşe İdil Çakıroğlu, Onur Kerimoğlu, Nihan Yazgan, Özge Karabulut, Arda Özen, Korhan Özkan ,Eti Levi. I will always remember the moments that we shared. We were the best limnology lab team ever.

My lab-mate, homeowner, mentor and an indispensable part of my life, Sara Banu Akkaş. From the first day that I came to Z-55 till now, we lived through so much together and managed to overcome every difficulty eventually. Thank you for everything!!!

My special, adorable friends, Gülşah Fidan, Havva Dinç, Kubilay Yıldırım, Oya Ercan ,Özge Selçuk, Didem İkis, Emre Yücetürk, Ömer Mescigil, İnci Narin, Alper Aygar, Görgülü Couple and Murat Yıldırım, you all were always there for me and without your support, love and patience (I knew that this was the hardest part), none of this could be possible. Love you so much.

At last, I would like to thank to my family; Zeynep, Ergin, and Didem Bezirci. Their unconditional love, continued encouragement, support and belief in my abilities nurtured me through this process.

This study was supported by Scientific and Technical Research Council of Turkey (TUBITAK) under the grant number 104Y308.

TABLE OF CONTENTS

LIST OF TABLES

TABLE

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Importance of *Daphnia Pulex* in Freshwater Ecosystems

In lakes and ponds cladocerans especially species belong to *Daphnia* (Figure 1.1) genus act as a link in the food chain: most of them are herbivorous, feed on phytoplankton and, in turn, are preyed upon by certain invertebrate and fish predators (Sarma et al., 2005). Thus, they play an important role in the transfer of energy from primary producers to higher trophic levels in freshwater food-web (Figure 1.2) (Dodson and Frey, 2001; Dumont and Negrea, 2002). Furthermore, daphnids serve as model organisms for not only understanding the life history parameters but also comparing the strategies under different food, predation, and temperature conditions as well (Gliwicz, 2003). Those properties that make *Daphnia* as suitable model organism are:

• *Daphnia* species are geographically widespread and distributed worldwide, which makes them interesting for studies of dispersal and phylogeography.

• They reproduce fast and with direct development (i.e. no free larval sta ges), hence the generation time is short, large populations can be produced in short periods, and populations respond quickly to environmental changes.

• They are easy to culture.

• They are rather transparent; hence the function of inner organs is visible from outside, which makes them particularly suitable for morphological and physiological studies.

• They carry their developing eggs in a brood pouch, which makes calculation of life history and population dynamics parameters easy.

• They are small (mm range) but large enough to be handled individually, hence they can be used in microcosm and mesocosm experiments.

• They reproduce mostly parthenogenetically. Clones can be kept in the laboratory for extended periods. Population genetic studies can study clones as units of selection.

• They are cyclic parthenogens, i.e. they can reproduce sexually under certain conditions. Products of sexual reproduction are dormant eggs encased in a durable ephippium that can survive harsh conditions for many years and function as a dispersal agent. This permits the study of the consequences of asexual and sexual reproduction.

• They occupy a central position in aquatic food webs, consuming primary producers and being consumed by predators. Hence they can be used to study interactions between trophic levels. In many lakes they are quantitatively very important and can be considered keystone species.

• This central position makes them ideal objects to study adaptations to multiple interactions within a food web.

• They are accessible for mathematical modeling on various levels, as their physiology, populations dynamics and ecosystem impact have been well studied.

• Their phenotypic and genotypic variability, and their phylogeny are well described (Lampert, 2006).

Figure 1.1 *Daphnia pulex*, photographed by Gizem Bezirci at METU Limnology Laboratory in 2007.

Using their strong grazing capacity, *Daphnia* individuals not only enhance the water clarity by feeding on phytoplanktons but also they controlled the - phytoplankton growths (Schriver *et al*., 1995; Jeppesen *et al*., 1999; Scheffer, 1999). As a results of their strong grazing capacity, the clarity of water is enhanced and this makes the opportunity for macrophytes to grow which are very important refuges for many aquatic organisms.

Daphnia has an important role in the protection of water clarity and as a result of protection of biodiversity in lake ecosystems (Edmondson and Litt, 1982). Consequently, the factors which will affect *Daphnia* populatin, will directly affect the water clarity and diversity in aquatic environment. So

using this information, *Daphnia* can be used as a biomarker which will help the ecological assesment of freshwater ecosystems.

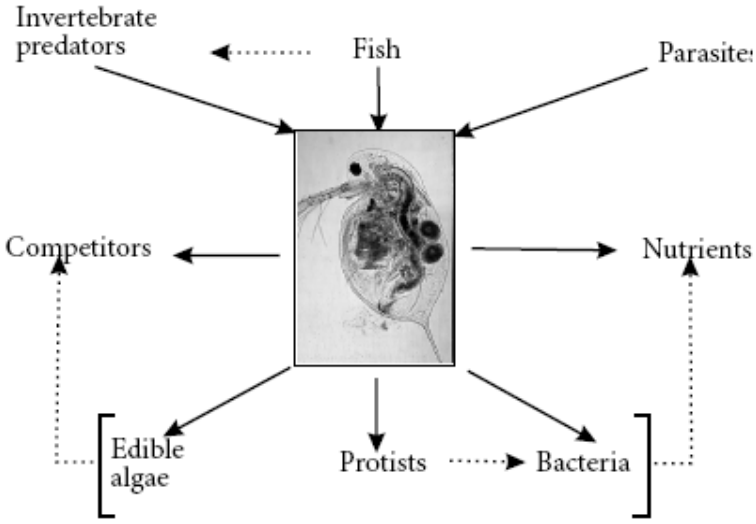

Figure 1.2 The central position of *Daphnia* in pelagic food web and it's interactions with other web components (Lampert, 2006).

1.2 Environmental Factors Effects *Daphnia* Survival and Life History Traits

Natural and anthropogenic stressors can affect ecosystem functioning through changes in biodiversity, especially when ecosystem processes (e.g. primary production) are maintained by only a few species (Tilman, 1999; Vinebrooke, *et al*., 2003).

Daphnia have such an important position in the freshwater food web and under optimum conditions, *Daphnia* individuals use %27 of their energy for assimilation, %68 for reproduction and %5 for growth (Richman, 1958).

Changes in the environmental conditions (predation pressure, increased salinity, temperature alteration, lower food availability, etc.) can change energy budget of the daphnids and will directly affect the survival and life history traits of daphnia individulas.

1.2.1 Impacts of Temperature

Abiotic factors such as temperature are important to most organisms (Andrewartha and Birch, 1984; Johnston and Bennett, 1996). Temperature is one of the major regulating factors in aquatic ecosystems through its impact on rate-dependent processes (Goss and Bunting, 1983; Beisner, McCauley and Wrona, 1996; Moore, Folt and Stemberger, 1996). Water temperature is a critical abiotic factor that effect reproduction and survival of whole food web especially enhancing predators growth e.g. fish (Beamish, 1995; Magnuson, Meisner and Hill, 1990; Shuter and Post, 1990; de Stasio *et al*., 1996), by affecting reproduction, e.g. in Arctic charr (Gillet, 1991) or by changing the timing of resource peaks (Straile, 2002).

The effects of temperature on life history (Ward and Stanford, 1982; Orcutt and Porter, 1983), behaviour (Ward and Stanford, 1982; Haney, 1993) and physiology (Simcic and Brancelj, 1997; Arscott, Bowden and Finlay, 2000) of freshwater organisms are well-documented. Through its impact on the activity of individual organisms, temperature potentially alters biological interactions and community structure (Moore and Folt, 1993; Beisner *et al*., 1996). Temperature can affect the outcome of predator– prey interactions (e.g. Dodson and Wagner, 1996), for example, via its influence on predator feeding rate (Beisner *et al*., 1996) or through effects on prey body size, which is important for size-selective predators (Moore *et al*., 1996).

A strong interaction between effects of temperature and salinity on survival in *Daphnia magna* has been demonstrated, a high temperature compounding the harmful effect of the salinity (Casey *et al.,* 2000). Temperature may also influence fish–*Daphnia* interactions indirectly, by affecting the chemical signalling that plays a crucial role in this predator–prey system. (Lass and Spaak, 2003) *Daphnia* can sense the presence of fish through chemical cues, so-called kairomones, and respond with changes in morphology, behaviour and life history (for review see Larsson and Dodson, 1993; Tollrian and Dodson, 1999). These anti-predator defences are not simple on / off-reactions but are adjusted to the prevailing predation risk (Tollrian, 1993; Loose and Dawidowicz, 1994; Reede, 1995; von Elert and Pohnert, 2000). Temperature also influences the strength of the response to fish kairomones (Havel, 1985). It might be advantageous for prey to exhibit stronger antipredator defences at high temperature, because the feeding activity of predators is temperature-dependent (Becker, 1992; Mehner, 2000).

1.2.2 Impacts of Salinity

Natural changes, such as the seasonal evaporation of water from freshwater bodies, can contribute to an increase in the salinity of many aquatic ecosystems (Das *et al*., 1995; Williams *et al,*. 1998). Among the abiotic factors influencing the survival and abundance of zooplankton inhabiting temporary water bodies, salinity is one of the most important (Williams, 1987; Dodson and Frey, 2001; Wallace and Snell, 2001). Since saline and freshwater environments require completely different adaptations if the animals inhabiting them are to retain suitable osmotic pressure and cell homeostasis, most aquatic organisms are unambiguously characteristic of either one habitat or the other (Young *et al,.* 1989).

Nevertheless, salinity varies markedly in many habitats, such as estuaries or coastal lakes (Hall and Burns, 2002; Schallenberg *et al.,* 2003).

Recently increasing salinity become a major problem for all freshwater ecosystems. Especially from 2002, the salinity levels of Lake Eymir and Mogan which were located in Middle Anatolia, were increased significantly as a result of decrease in the lake levels and increased retention time and from these observations it could be anticipated that salinity could be a major stress factor for most of the lakes in Turkey (Beklioğlu and Özen, 2007). Because of the effects of the osmoregulatory activities, salinity is an important stress factor for the freshwater organisms (Baillieul *et al.,* 1996). Its level and variations have an impact on the composition and osmolality of the body fluids of animals, among which some groups or species possess physiological abilities of osmoregulation (Charmantier and Daures, 2001). Even though freshwater invertebrates have been submitted over a long period of time to selection to cope successfully with the low osmotic pressure of their present habitat (Grzesiuk and Mikulski, 2006) , they seem to be amongst the most sensitive freshwater animals to increases in salinity, with adverse effects apparent for some species at salinities as low as 1‰ (Hart *et al*., 1991) and even minor saline intrusions can also result in severe perturbations of zooplankton community structure and abundance (Schallenberg *et al.,* 2003).

Zooplankton groups, particularly rotifers, cladocerans and copepods, represent the dominant component in freshwater bodies and are the natural food link between the primary producers (algae) and zooplanktivorous fish (Sarma *et al*., 2006). As such they are important in the maintenance of an ecological balance in freshwater ecosystems (Nogrady *et al*., 1993). Unlike copepods, which are dominant in marine systems, rotifers and cladocerans are largely restricted to freshwater habitats (Sarma *et al*., 2006).

In addition, both of these groups are generally very sensitive to salinity (Dodson and Frey, 2001; Wallace and Snell, 2001). Thus, an increase in salt levels in freshwater ecosystems affects the dynamics and abundance of rotifers and cladocerans (Akopian *et al*., 2002). Also, under stressful conditions, including salinity, the growth rates of freshwater zooplankton can be negative (Sarma *et al*., 2002). These studies show that total zooplankton (Frey, 1993; Green, 1993; Hammer, 1993; Jeppesen, et al., 1994; Garcia, et al., 1997; Ramdani, et al., 2001a), cladoceran (Frey, 1993; Green, 1993; Ramdani, *et al*., 2001), rotifer (Green, 1993; Green and Menengestou, 1993), and copepod (Green, 1993; Ramdani, *et al*., 2001) taxonomic richness all decrease with increasing salinity.

Zooplankton have different tolerance levels to salinity (Aladin, 1991). Increased salinity lead to increase in mortality of daphnids (Teschner, 1995; Hall and Burns, 2002). Even where it does not reduce lifespan of the animals, salinity may limit individuals' growth rates, with freshwater animals transferred to a brackish environment found to grow more slowly (Hall & Burns, 2002; Teschner, 1995; Arner and Koivisto, 1993) and can cause both delayed maturity and a smaller size at first reproduction (Teschner, 1995). Large-sized *Daphnia*, which are the main controllers of phytoplankton in freshwater lakes (Carpenter and Kitchell, 1993), appear mainly at relatively low salinity (<2&) (Jeppesen *et al*., 1994), an exception being *D. magna*, which tolerates higher salinity (Ortells *et al*., 2005). Salinity-dependent modifications to the life histories of freshwater crustaceans (as described below) suggest the possibility of salinity-dependent modifications to physiological processes, though the relevant data are again scarce and limited to those described by Arner and Koivisto (1993). These authors suggest that respiration and ammonium-excretion rates in *Daphnia magna* Strauss are lowest where salinity is optimal. Active regulation of internal salinity under the influence of too high or too low salt

concentration entails an increased respiration rate, since osmotic regulation is associated with increased metabolic rate.NaCl is commonly found in freshwater ecosytems as a salinity source and ionic acitivies of Na^+ and Cl^- accomadates the osmotic balance for the organisms. For the crustaceans, alteration of the ionic composition and loss of osmotic regulation mechanism were mostly related with the changes in the ionic compositions of Na^+ and Cl^- (Lignot, 2000; Heugens, 2003).

The use of freshwater zooplankton for evaluating the impact of increased salinity levels has some relevance to the management of freshwater bodies and aquacultural ponds (Sarma *et al*., 2006). It permits the limits of salt tolerance to be established so that remedial measures can be taken to protect freshwater waterbodies from salinization (Peredo-Alvarez *et al*., 2003).

1.2.3 Impacts of Food

Imbalances between food resource and consumer demands can decouple the flow of energy in food webs by reducing the efficiency of zooplankton growth, as has been shown in a multitude of studies, (Hessen, 1992; Gulati and DeMott, 1997; Andersen *et al*., 2004). A lower food quality can generally be caused by ingestibility (DeBernardi and Giussani, 1990), stoichiometric imbalance (Sterner and Schulz, 1998; Brett, Müller-Navarra and Park, 2000), lack of essential biochemical substances (Mü ller-Navarra, Brett and Liston, 2000; Martin-Creuzburg *et al*., 2005), or toxicity (Wilson, Sarnelle and Tillmanns, 2006).

Food quantity and quality are of main factors constraining zooplankton influencing reproduction, survival, and competitive mechanisms (Lampert, 1985). Most cladoceran species are generalists in their feeding mode and

are thus adversely affected by extremely high algal concentrations (Downing and Rigler, 1984). Because food quality strongly influences life-history parameters of *Daphnia* this should also influence the experienced trade-off between temperature and food, provided *Daphnia* are able to measure food quality (Reichwaldt, 2008).

Warmer summers with little vertical mixing provide ideal conditions for surface blooms of harmful cyanobacteria (Reynolds, 1997; Ibelings *et al*., 2003; Robson and Hamilton, 2003; Mooij *et al*., 2005) which will be a limiting factor for *Daphnia* through reducing somatic growth, fecundity and reproductive ratedue to poor food quality and quantity (Lampert *et al*., 1986, Urabe *et al*., 1997; Ferrao-Filhoand Azevedo, 2003). Lampert (1977a) observed a linear increase of filtering rates by *Daphnia pulex* under low food concentrations until a plateau was reached, known as incipient limiting level (ILL). Below the ILL, food concentration could be the main factor influencing *Daphnia* production (Lampert 1977b).

1.2.4 Impacts of Predation Pressure

In aquatic environments, predation is one of the most important factors influencing community structure and the evolution of life-history characteristics of zooplankton (Lynch, 1980; Zaret, 1980; Kerfoot and Sih, 1987). Invertebrate and vertebrate predators commonly prey upon zooplankton, and both types of predators exhibit significant differences in selectivity based on prey size (Dodson, 1974; Zaret, 1980).

The chemicals released from predators have been called kairomones, defined as chemicals giving benefit to receivers rather than releasers (Brown, *et al*., 1970) and they are predator-specific, and allow zooplankton to detect potential predators first, and then to allocate energy

differentially towards morphology, growth, and reproduction to reduce immediate predation risks (Tollrian and Dodson, 1999).

Anti-predator defences are effective in reducing mortality (Havel and Dodson, 1984), but they are typically associated with a reduction in reproductive output (Kerfoot and Sih, 1987). Therefore, anti-predator defences are often inducible and only employed in the presence of a predator (Tollrian and Harvell, 1999; Lass and Spaak, 2003). In aquatic systems, zooplankton can mitigate the impacts of predators by behavioural, morphological and life-history responses that are triggered by a chemical cue (kairomone) exuded by the predator (De Meester *et al*., 1999; Tollrian and Dodson, 1999). These responses either reduce predation or allow high population growth to compensate for high predation (Zaret and Suffern, 1976; Stich and Lampert, 1981; Lampert, 1993).

When visually feeding fish are abundant, the herbivorous cladocerans *Daphnia* spp. often react by migrating downward into darker and colder water layers during daytime (Ringelberg, 1991; van Gool and Ringelberg, 1995). Egg development time increases at colder temperatures (Bottrell, 1975), which results in a major reduction in population growth rate in the case of vertical migration (Dawidowicz and Loose, 1992; Boeing, 2002). Other defences by *Daphnia* against fish include lower growth rate, reduce the number of juvenile instars, period of carrying eggs in brood chambers, number and size of neonates, for example produce larger clutches of smaller offspring earlier, or morphology (Havel and Dodson, 1987; Riessen and Sprules, 1990; Black and Dodson, 1990; Spitze, 1991; Hanazato and Dodson, 1992) Machacek, 1991; Stibor, 1992; Weider and Pijanowska, 1993; Mikulski, 2001; Sakwinska, 1998 and 2002.

Morphological defenses such as body shape changes (Brönmark and Miner 1992; Kuhlmann *et al*., 1999; Van Buskirk and Schmidt 2000) and the growth of defensive spines (Krueger and Dodson 1981; Harvell 1986; Havel

and Dodson 1987) that reduce predation rates. Thus, such changes in lifehistory characteristics are considered to be negative responses by *Daphnia* to the kairomone.(Hanazato, *et al*., 2001)

1.3 Impacts of Global Warming on Freshwater Ecosystems

The Earth's climate is determined by a number of complex connected physical, chemical and biological processes occurring in the atmosphere, land and ocean (IPCC, 2007). In this century, the warming is projected to continue at a rate somewhat greater than its global mean, with the increase in 20-year mean temperatures (from its values in 1980 to 1999) becoming clearly discernible within a few decades (IPCC, 2007). Climatic forcing is increasingly recognized as a potential factor which changes food webs, especially if interacting species respond differently to altered environmental conditions (DeStasio *et al*., 1996; Petchey *et al*., 1999; Straile, 2000; Talling, 2003; Winder and Schindler, 2004; Emmerson *et al*., 2005).

Of all ecosystems, freshwater ecosystems will have the highest proportion of species threatened with extinction due to climate change (Millennium Ecosystem Assessment, 2005) and climate is a major factor responsible for long-term changes of thermal properties and biological processes in freshwater ecosystems, if anthropogenic influences are absent (Carpenter *et al*., 1992). Many of the areas (e.g., Mediterranean basin, western USA, southern Africa, and north-eastern Brazil) will suffer a decrease in water resources due to climate change (very high confidence) (IPCC, 2007). Turkey, being located in the Mediterranean basin between 36-N and 42-N latitudes, lies on a very steep hydrological gradient in which runoff decreases very dramatically (IPCC, 2007).

Given the current climate change projections, climate change might be one more driving forces (Sala *et al*., 2000), leading to a direct loss of species when the environmental variables reach levels that are beyond what an individual species can cope with through acclimation and adaptation (Thomas *et al*., 2004). Biodiversity is expected to decline in freshwater habitats in response to climate change at a far greater scale than is true for even the most affected terrestrial ecosystems (Ricciardi and Rasmussen, 1999; Sala *et al*., 2000).

In marine and freshwater ecosystems, many observed changes in phenology and distribution have been associated with rising water temperatures, as well as changes in salinity, oxygen levels and circulation patterns (IPCC, 2007; Strecker *et al*., 2004;Gerten and Adrian, 2002; Winder and Schindler, 2004, Ahas, 1999; Elliott *et al*., 2000, Wagner and Benndorf, 2007). Beisner *et al*. (1997) found that a predator– prey equilibrium could be rapidly destabilised with an increase in temperature in a system with three trophic levels, including an invertebrate predator, which was dynamically coupled to a herbivore. Those changes and interactions between populations are crucial to the estimation of the impact of global warming on aquatic food webs (Wagner and Benndorf, 2007). And timing of interactions between populations and the resulting consequences for population dynamics and ecosystem processes have emerged as central mechanisms of how global change will modify the biotic structures of ecosystems (Harrington *et al*., 1999; Visser and Both, 2005).

1.4 Scope of The Study

Understanding multiple stressors is particularly challenging when their combined effect cannot be predicted based on evidence from single-stressor studies _/ i.e. there are interactions that cause non-additive effects (Breitburg, *et al*., 1999, Folt, *et al*., 1999). Stressors are synergistic when their combined effect is larger than predicted from the sizes of the responses to each stressor alone, and antagonistic when the cumulative impact is smaller than expected (Folt, *et al*., 1999). Given the increasing multiplicity of environmental stressors associated with global change, there is an urgent need to develop a better understanding of the interactive effects of multiple stressors on ecosystems to better predict their responses to a changing environment (Vinebrooke, *et al*, 2004).

Impacts of salinity and fish-exuded kairomone on the survival and life history traits of *Daphnia* have not been studied before. In this study first we took the advantage of such gap in the literature to explore the impacts of those natural stressors together, than by adding stressors like temperature and food limitation, tried to obtain a more realistic approach to the responses of daphnids to changes in the environmental conditions.

CHAPTER 2

MATERIALS AND METHODS

2.1 Preparation of Climate Room and Live Cultures

2.1.1 Preparation of Climate Room

To cultivate the *Daphnia* and algal cultures at Middle East Technical University, Biology Department, there are two climate rooms which contain ''Arçelik 4040S Inverter split'' air conditioner and ''Denco Air Conditioning'' air conditioning system. With those air conditioners, the temperature was stabilized to 22±1 ºC. Those two rooms also include special lighting equipment which are connected to the main lighting system and can arrange room lighting conditions to 16h light: 8h dark regime.

2.1.2. Preparation of Live Cultures

2.1.2.1 Preparation of *Scenedesmus obliquus* Culture

Daphnia cultures were fed with pure and live *Scenedesmus obliquus*. (Pure culture: Gottingen University, Alg Culture Centre). The procedure for preparing of *Scenedesmus obliquus* culture medium was shown in Table 2.1. The solid algal cultures were prepared with the addition of 15 g agar into the culture medium. Algal cultures were cultivated into the climate room in sterile contiditons with special lighting system. In order to prevent them sink, cultures were continuously stirred up on magnetic stirrers (Figure 2.1).

Figure 2.1 *Scenedesmus obliquus* culture in the laboratory (METU Limnology laboratory - Z-55).

Table 2.1 Solutions for preparation of *Scenedesmus obliquus* culture Media.

Proteose Peptone Media		
To 940 mL of glass-distilled water, , add 1.0 g Proteose peptone and the following solutions		
mL	**Stock solution**	**g/400 mL Dh_2O**
10	$NaNO_3$	10.0
10	$CaCl_2 \cdot 2H_2O$	1.0
10	$MgSO_4 \cdot 7H_2O$	3.0
10	K_2HPO_4	3.0
10	KH_2PO_4	7.0
10	NaCl	1.0

To determine the required amount of food for *Daphnia*, Chlorophyll-a content of algal culture was determined using a method described by Jespersen and Christoffersen (1989). Ninety ml of dechlorinated distilled was added to the 10 ml liquid algal culture and filtered through 47 mm Watman GF/C filter paper. The filter paper was then placed in a centrifuge tube and incubated in 10 ml of ethanol (%96) and left in cold and complete dark conditions for 12 hours. Then the sample was centrifugated in 4000 rpm for 20 minutes in 4 °C. The absorbance of the sample was measured at 750nm and 663nm against ethanol blank. The chlorophyll-a concentration was calculated according to the equation below:

$$\text{Chlorophyll-}_{a}\,(\mu g/L) = [11.0x\,(A_{663}-A_{750})xV_{ethanol}]/V_{filtered\ water}$$

A_{663}: The absorbance at 663nm

A_{750}: The absorbance at 750nm

$V_{ethyl\ alcohol}$: The volume of ethanol blank in mL

$V_{distiled\ water}$: The volume of filtered water in L

Then the concentration of chlorophyll-a was converted to C amount using the conversion factor of 30 mg C: 1 mg chl-a 1^{-1} suggested by Reynolds (1984). The C amount in the experimental vessels was adjusted to 1 mg C^{-1}, 0.400 mg C^{-1} and 0.075 mg C^{-1} as in the necessary experiments.

2.1.2.2 Preperation of Fish Culture

In order to prepare fish exuded kairomone, medium sized (10-15 cm) *Alburnus alburnus* caught with 200m nets contained 10 mm mesh size from Lake Eymir were used. Fish were kept alive in the aquariums in the climate room. The water in the aquariums renewed once a week and the fish were fed everyday with synthetic food. Throughout the experimental process, two medium-sized fish were put into 10L of COMBO medium and incubated there at least 14 hours to obtain fish-exuded kairomone (Loose *et al*., 1993; Loose and Dawidowicz, 1994). Then fish was taken away from the COMBO medium and medium were filtered with first 0.45µm mesh sized than 0.22µm mesh sized filters. Lastly, the pH was adjusted to 7.50±0.05. Finally, the medium contains fish kairomone and was ready to use in the experiments.

2.2 Preparation of Culture Conditions

2.2.1 COMBO Culture Medium

In order to conduct experiments on interactions between the test animals and food organisms, it is necessary to develop a medium that adequately supports the growth of both algae and zooplankton without needing to alter the medium to accommodate either the algae or the test animals (Kilham *et al*., 1998). For that reason, COMBO culture medium, which meets the criteria, was selected to rear *Daphnia for the* experiments (Kilham *et al*., 1998). COMBO culture medium is commonly used in the toxicology studies and in the literature it's commonly accepted to be the proper culture medium for zooplankton species (Kilham *et al*., 1997a; Kilham *et al*., 1997b; Urabe *et al*., 1997; Schallenberg *et al*., 2005).

Furthermore using the defined culture media provides the compatibility between experiments and avoids the effects of the chemicals and pollutants that may likely to be found in the lake water (Hall and Burns, 2002).

COMBO culture medium was always freshly prepared before the experiment. It was prepared by adding the followings a) 1 ml of each of the seven major element working solutions, b) 1 ml of ATE, c) 1 ml of ANIMATE, d) 0.5 ml of Vitamins (VIM) to 750 ml distilled and deionized water (LPW) (Kilham *et al*., 1998). Then the medium was filtered through 0.22µm or 0.45µm. The medium pH was adjusted to 7.50±0.05. The procedure for preperation of the COMBO culture medium is also shown below Figure 2.2.

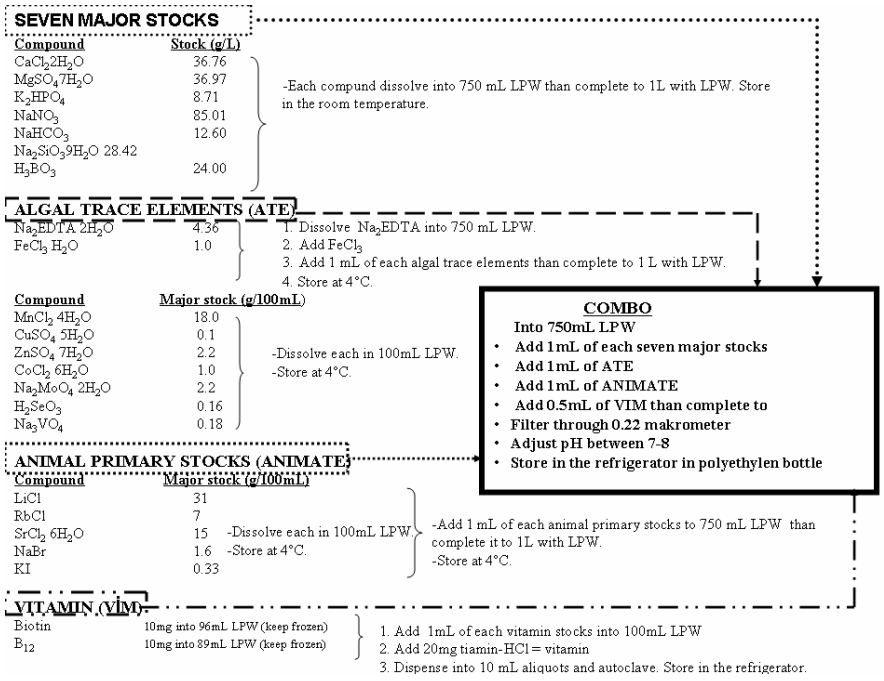

Figure 2.2 Procedure for preparation of COMBO culture medium (Kilham et al., 1998).

2.2.1.1 Acclimation *Daphnia pulex* to The COMBO Medium

Acclimation is a time dependent process (Vijverberg, 1989). Acclimation of zooplanktoners to temperature, food and salinity were already carried out by several of researchers (Munro, 1974; Hart and McLaren, 1978; Vijverberg, 1989; Arner, 1993; Baillieul *et al*., 1996; Martinez *et al*., 2007). In the acclimation studies to eliminate the maternal effect, zooplankton cultures have to grow at least two generations in the same conditions. After that process newly born neonates can be used in the experiments. Such procedure is commonly applied in the literature (Hrbacek, 1977; Goulden *et al*., 1982; Vijverberg, 1989; Arner, 1993; Baillieul *et al*., 1996; Martinez *et al.,* 2007).

A clone of *Daphnia pulex* were collected from Lake Eymir, Ankara and they were cultured in filtered and conditioned lake water in the climate room (22±1 ºC and 16 h light :8 h dark regime) for several years. For the experiments presented in this study, COMBO medium was selected to rear *Daphnia* clones that they were acclimated to the combo. This acclimation procedure took three months. To gain individuals which all have the same genetic structure, *Daphnia* individuals monitored at least three generations and egg bearing individuals' separated as a part of this process (Figure 2.3). Through this selection procedure not only maternal affect was eliminated but also individuals were adapted to the COMBO culture medium. To avoid the crowding effect, no more than 100 individuals were put in 1 L COMBO culture medium and as the specimens grew, individuals were continuously transferred to new mediums. In the experiments, neonates of the second generation were used (Doksæter and Vijverberg, 2001).

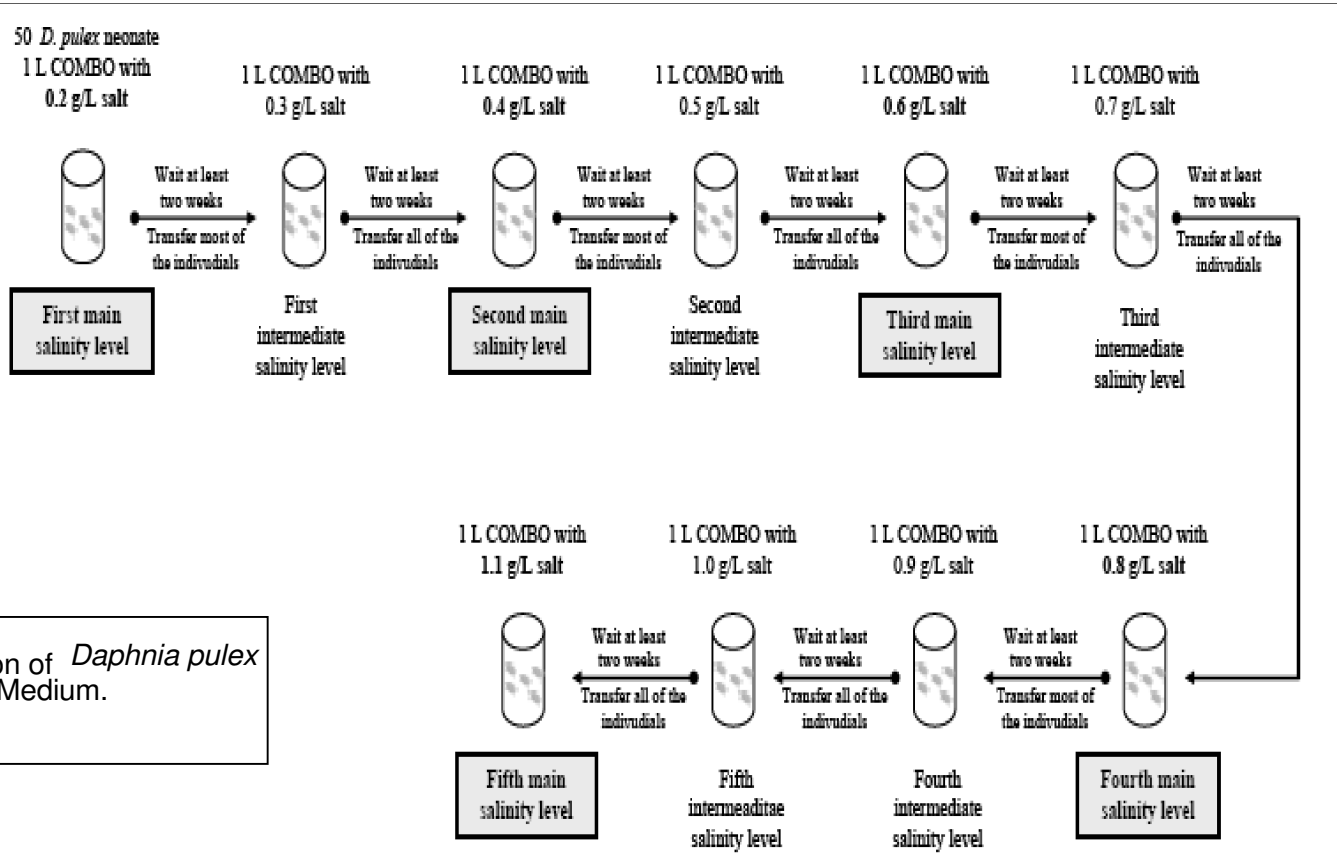

Figure 2.3 Adaptation of *Daphnia pulex* to COMBO Culture Medium.

2.3. Toxicity Experimental Procedures

2.3.1 OECD Test Guideline

All acute and chronic toxicity experiments were carried out following the OECD Test Guideline (1984 and 2004).

A) Acute Toxicity Experiments

Description of the acute test procedure is according to the OECD guideline (1984 and 2004):

Equipment which will come into contact with the test solutions should preferably be all glass; the glassware should be cleaned with solvents known to remove previously tested chemicals.

- Individuals of *Daphnia* used for the experiment should be not more than 24 hours old at the beginning of the test.
- At least 20 animals, preferably divided into four groups of five animals each, should be used at each test concentration and for the controls.
- The *Daphnia* should not be fed during the test.
- Loading: at least 2 ml of test solution should be provided for each animal.
- The test temperature should be between 18 and 22 ℃, and for each single test it should be constant within ±1℃.
- To avoid the necessity of adaptation prior to the test, it is recommended that the water used in the test be similar to the culture water.

Also immobilisation means that the test animals not being able to swim within 15 seconds after gentle agitation of the test container (OECD, 1984 and 2004). During the experiments survival of the animals and 24-48h EC_{50} values were recorded and estimated, respectively.

B) Chronic Toxicity Experiments

According to OECD guidelines (1984, and 2004), the objectives and the test requirements as follows: the primary objective of chronic test is to assess the effect of toxicant on the reproductive output of the test organisms such as *Daphnia pulex.* The survival of the parent animals and time to production of first brood must also be reported.

Conditions for chronic test as follows:

- Neonate female *Daphnia*, aged less than 24 hours at the start of the test, are exposed to the test substance added to medium at a range of concentrations.
- Daily, survival of the test organisms and the total number of living offspring produced per parent animal alive at the end of the test are assessed.
- There should be at least five test concentrations arranged in a geometric series with a separation factor preferably not exceeding 3.2, and the appropriate number of replicates for each test concentration should be used.
- If ECx (the concentration of the substance dissolved in water that results in a x percent reduction in reproduction of *Daphnia magna* within a stated exposure period) for effects on reproduction is estimated, it is advisable that sufficient concentrations are used to define the ECx with an appropriate level of confidence**.** If the

EC_{50} for effects on reproduction is estimated, it is advisable that the highest test concentration is greater than this EC_{50}.

- Those animals not able to swim within 15 seconds after gentle agitation of the test container are considered to be immobile (OECD, 1998).

Description of the test procedure according to the OECD guideline:

Equipment which will come into contact with the test solutions should preferably be all glass; this glassware should be cleaned with solvents known to remove previously tested chemicals.

- *Daphnia* species, not more than 24 hours old at the beginning of the test.
- The test duration is 21 days.
- For semi-statistic test, the number of animals to be used at each test concentration has been reduced from at least 40, preferably divided into four groups of 10 animals, to least 10 animals held individually.
- Feeding should preferably be done daily. The food ration should still remain within the recommended range of 0.1 - 0.2 mg C/*Daphnia*/day at all times.
- At least 2 ml of test solution should be provided for each animal.
- The frequency of medium renewal will depend on the stability of the test substance but should be at least three times a week.
- When the medium is renewed, a second series of test vessels are prepared and the parent animals transferred to them.
- Mortality among the parent animals should be recorded preferably daily.
- The test temperature should be between 18 and 22℃, and for each single test it should be constant within ±1℃.
- To avoid the necessity of adaptation prior to the test, it is recommended that the water used in the test be similar to the culture water.

2.4 Experimental Designs

2.4.1 Salinity Toxicity Experiments

Daphnia pulex individuals which were grown in the COMBO culture medium were followed for at least two generations, were then used in the acute and chronic toxicity experiments. Experiments were held in the climate room under the 22±1 ºC and 16h light: 8h dark regime conditions.

Salinity was chosen as a stressor as it is a natural stressor to which animals can, within limits, , become fully acclimated by altering their osmotic and ionic regulation (Baillieul et. al 1996) There is some debate on the validity of using pure NaCl as the source of salinity when investigating organisms collected from bodies of water with different ionic compositions (Kefford et al. 2004). However, Sarma et al. (2005a) reported that invertebrate organisms such as rotifers that inhabit temporary water bodies in which high salt concentrations are the result of different ionic composition were able to tolerate the same levels of salinity based on NaCl concentration. According to the information in the literature and considered conditions of our freshwater ecosystems, NaCl had chosen to be the salinity source for the experiments.

2.4.1.1 Acute Toxicity Experiments

Acute toxicity experiments were performed in accordance with the standard protocol of OECD (1984). To detect the effects of NaCl on the survival of *Daphnia pulex*, three acute toxicity experiments were carried out. Salinity acute experiments were wisely applied in the literature for testing the effect of chemical of interest (Weider, 1993; Kefford, 2004 and 2007; Mohammed, 2006; Martinez and Martinez, 2006). It was the first time in the literature that salinity and fish predation used together as stressors. So the acute experiments were designed to determine LD_{24} and LD_{48} using doses through 10.0 g/L – 0.0 g/ L salt.

A) Toxicity Experiments: The highest Doses

This experiment was designed to determine the maximum resistance of *Daphnia pulex* to the highest salinity levels. Animals which were used in the experiments were at least second brood neonates and $\leq$48h-old. Individuals were put to beakers which contained 25 ml of the test media (COMBO medium) with 5 individuals per 4 replicates and were placed under controlled climate room with the temperature of 22±1 ºC and 16h light: 8h dark regime. The doses of NaCl used in the experiment were 0.0, 2.0, 4.0, 6.0, 8.0 and 10.0 g/L in the presence and absence of fish kairomone (Figure 2.4). The animals were not fed during the experiment. For 48 hours, survivals of the animals were examined periodically.

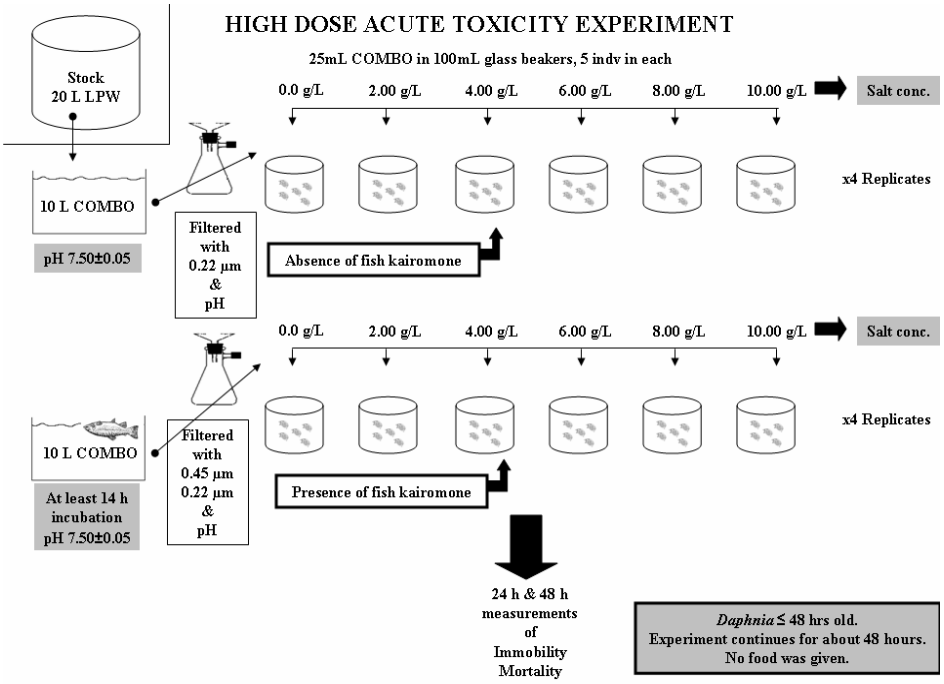

Figure 2.4 Diagram to show the high dose acute toxicity experiment.

B) Acute Toxicity Experiment: High Doses

In this acute toxicity experiment, the test organisms were exposed also high NaCl doses. But in the previous experiment it was shown that, the last two doses (8.00 and 10.00 g/L salt) were too high for *Daphnia* to survive, than in this experiment lower doses (lower than first experiment) with narrower ranges were chosen. Neonates were exposed to different concentrations of NaCl which are 0.0, 0.20, 0.60, 1.20, 2.25, 3.50, 5.00 and 6.50 g/L in the presence and absence of fish kairomone. Experimental procedure was the same as in ‘’First Acute Toxicity experiment: high dose‘’, explained above.

C) Acute Toxicity Experiment: Low Doses

In this acute toxicity experiment, the test organisms were exposed also different NaCl doses. After exploring having carried out two acute toxicity experiments with high doses, in this experiment, neonates were exposed to lower concentrations of NaCl which were 0.0, 0.05, 0.125, 0.25, 0.50, 1.25, 2.50 and 5.0 g/L in the presence and absence of fish kairomone. Experimental procedure was also same as above.

2.4.1.2 Chronic Toxicity Experiments

Chronic toxicity experiments were also performed with regards to the standard protocols of OECD (1984) to detect the effects of salinity, fish predation, temperature and food availability on the survival and life history traits of *Daphnia pulex*.

Three chronic toxicity experiments were carried out among which in one of the experiments, the effects of salinity, fish-exuded kairomone, temperature and food on survival of the specimens were determined and in another experiment the effects of salinity and fish predation alone on survival of the specimens were determined. These experiments were referred as “survival experiments”. In the third experiment, effects of salinity and fish-exuded kairomone on life history traits were examined. The details of the experiments are given below.

2.4.1.2.1 Survival Experiments

A) Effect of Salinity and Fish Predation on The Survival of *Daphnia pulex*

A test animal was placed to beakers which contained 40 ml of the test media (COMBO medium) with 10 replicates and they were kept under controlled conditions of the climate room (the temperature of 22±1 ºC and 16 h light: 8 h dark regime). Neonates were exposed to different concentrations of NaCl, which were 0.05, 0.10, 0.20, 0.40, 0.80 and 1.50 g/L in the presence and absence of fish-exuded kairomone (Figure 2.5). During the experiment animals were fed everyday with the appropriate amount of pure *S. Obliquus* culture to provide 1 mg C/L/day for *Daphnia* (Vijverberg, 1989). During the experiment in every third day the test solutions were renewed. Survival of the animals was checked every day.

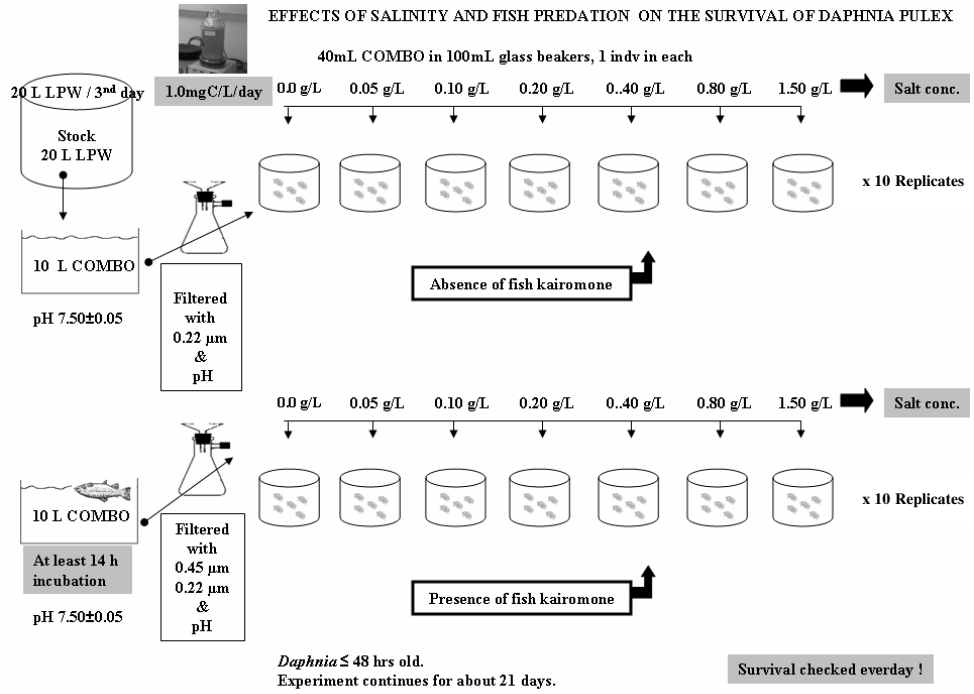

Figure 2.5 Effects of salinity and fish predation on the survival of *Daphnia pulex* chronic toxicity *test* experimental diagram.

B) Effects of Multitressors on The Survival of *Daphnia pulex*

Increase in water temperature, salinity, eutrophication in the aquatic systems are expected results of the global warming (IPCC, 2001 and 2007; Mooij *et al*., 2005; Kefford *et al*., 2004). Lakes Eymir and Mogan, located in the central Anatolian platue, have been monitored since 1997 by METU Limnology Laboratory, the data show that the salinity and nutrient levels of the lakes increased significantly (Beklioğlu and Özen, 2008). To determine the effects of multistressors including salinity, temperature, food availability and fish predation on the survival of *Daphnia pulex*, the following experimental setup was designed (Figure 2.6):

- Under two different temperature levels (Regular temperature: 22±1 °C and High temperature: 25±1 °C),
- Two different food levels (low food level: 0.075 mg C/L/d and optimum food level: 0.400 mg C/L/d),
- Presence and absence of fish kaioromone,
- Three different NaCl levels and control [0.00 (control), 0.10, 0.40 and 1.50 g/L]

During the experiment test solutions were renewed every third day and survival of the animals was checked daily.

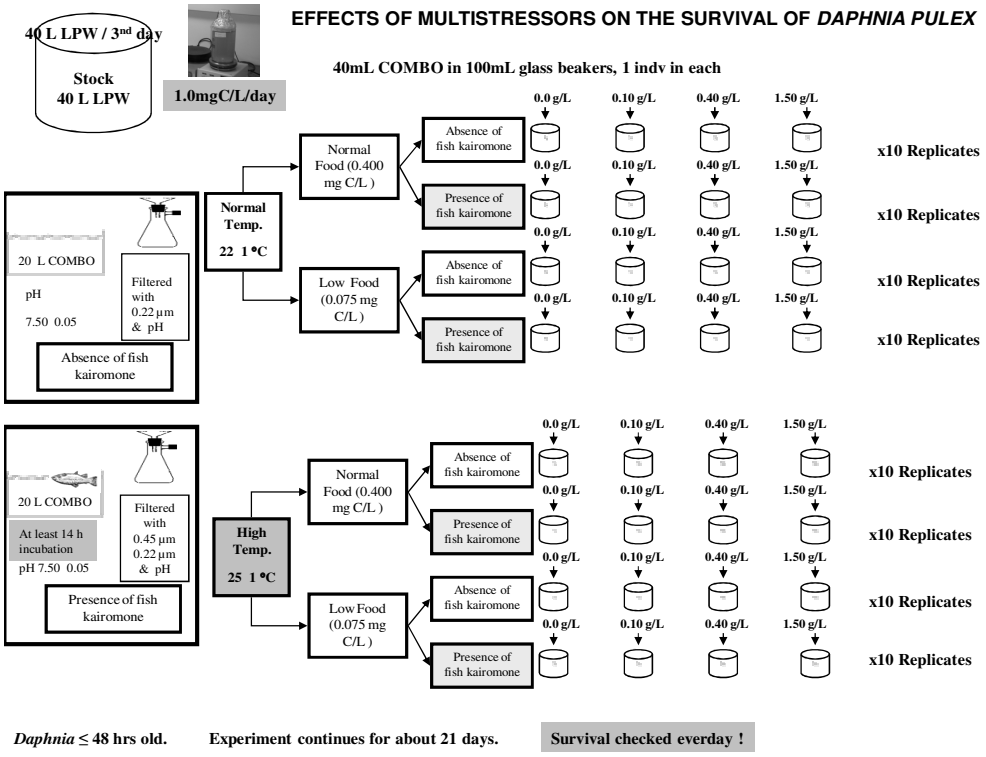

Figure 2.6 Effects of multitressors on the survival of *Daphnia pulex* chronic toxicity experimental diagram.

2.4.1.2.2 Life History Traits Experiment

Changes in the life histories of planktonic cladocerans constitute a well-known example of adaptive phenotypic plasticity (Riessen, 1999; Lass and Spaak, 2003). A potentially important plastic response to biological enemies is a shift towards more reproduction early in life, often termed fecundity compensation (Thornhill *et al*., 1986; Lüning, 1992; Adamo *et al*., 1995; Boersma *et al*., 1998; Polak and Starmer, 1998; Krist, 2001).

To be able study life history traits of *D. pulex*, the test animals were acclimated to the changing salt levels. In this study *Daphnia pulex* individuals firstly adapted to the COMBO culture medium (Figure 2.3). Later on they acclimated to different salinity levels (0.2, 0.4, 0.6, and 0.8, 1.1 g/L) to further use in the life history trait experiments.

To obtain salt-acclimated individuals (mostly neonates), first individuals were transferred into the COMBO medium which included 0.2 g/L of salt. This was the first main salinity level of the acclimation. *Daphnia* individuals lived in this salinity at least for two weeks which provided enough time to have at least two new generations (Vijverberg, 1989; Arner, 1993; Baillieul *et al*., 1996; Martinez et al., 2007). Afterwards, some of the individuals from the 0.2 g/L of salty media were transferred to COMBO media which included 0.3 g/L of salt. Individuals lived in this salinity level at least for two weeks then all of the individuals were transferred in to the COMBO medium which included 0.4 g/L of salt which was the second main salinity level of the acclimation procedure. As the procedure continued up until test animals were acclimated to 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, g /L salinity levels (Figure 2.7). Acclimation procedure was held in the climate room under the 22±1 ºC temperature and 16 h light: 8 h dark regime. Animals were fed daily with fresh *Scenedesmus obliquus* through the acclimation process.

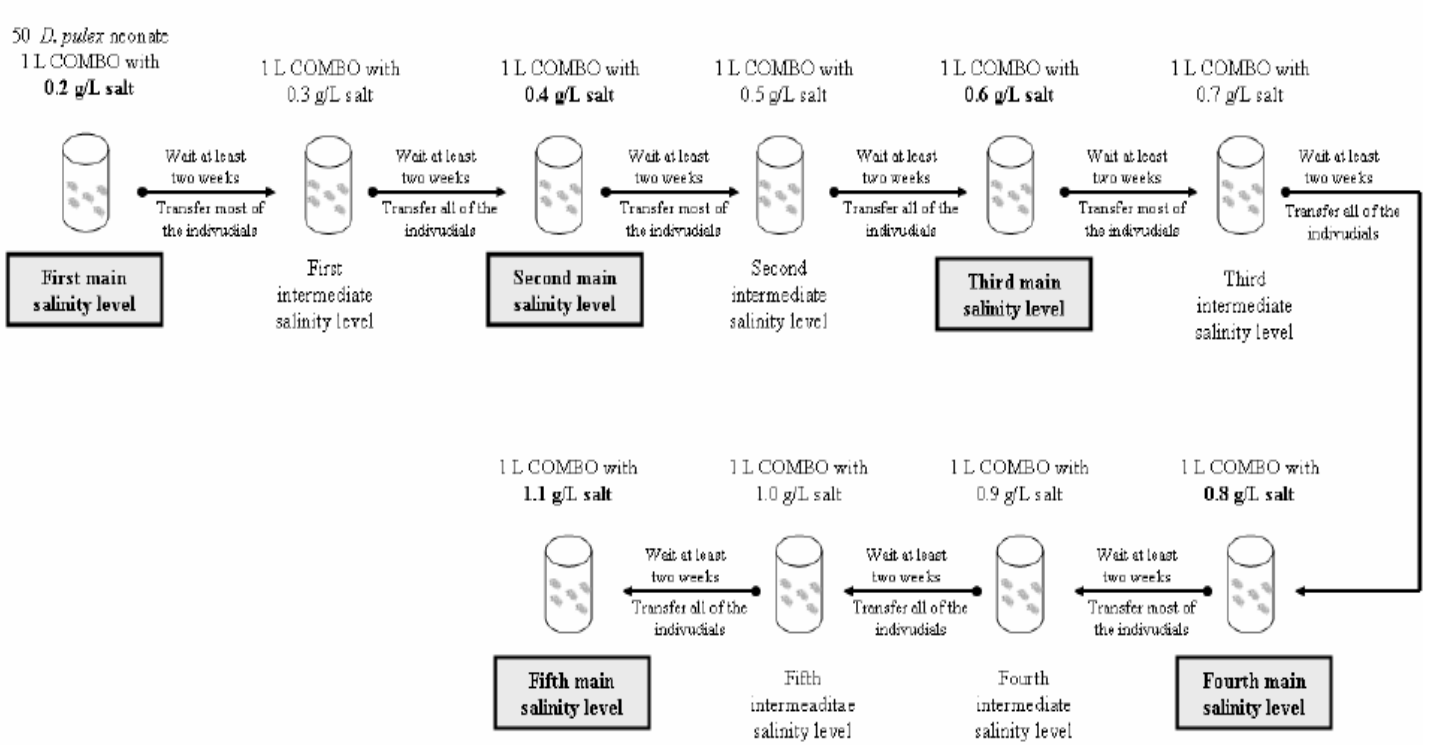

Figure 2.7 Acclimation process of *Daphnia pulex* to different salinity levels.

A) Influence of Salinity and Fish Predation on Life History Traits of *Daphnia pulex*

Animals which were acclimated to different salinity levels (0.2, 0.4, 0.6, 0.8, 1.1 g/L) were placed to beakers which contained 40 ml of the test media (COMBO medium) with 1 individuals per 10 replicates and were placed under controlled climate room with the temperature of 22±1 ºC and 16 h light :8 h dark regime. Neonates were exposed to different concentrations of NaCl which included 0.2, 0.4, 0.6, 0.8 and 1.2 g/L in the presence and absence of fish kairomone (Figure 2.8). Survival of the animals checked everyday. During the experiment animals were fed daily with pure *S. Obliquus* to 1 mg C/L/d for *Daphnia* (Vijverberg, 1989).

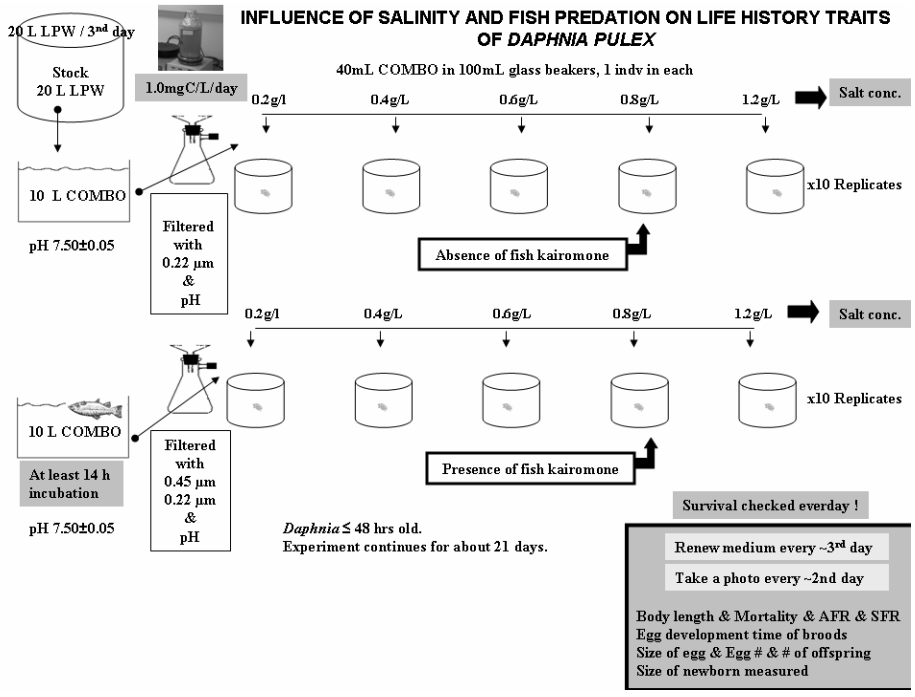

Figure 2.8 Influence of Salinity and Fish Predation on Life History Traits of *Daphnia pulex* chronic toxicity test experimental diagram.

During the experiment in every third day the test solutions were renewed and every second day all the animals were photographed using Leica M Stereo Microscope –MZ16 and attached DFC280 camera.

Animals were taken from the medium with plastic pipettes and placed on a petri dish, under the microscope and photographs were taken immediately (Figure 2.9). Using those photos, life history traits, which were, age and size at maturity (age and size at first reproduction) and at successive reproductions, the number of juvenile instars, period of carrying eggs in brood chambers and egg size, number and size of neonates or morpholologic changes like length and width of the adults and offsprings were measured (Figure 2.10). All measurements and photos were taken from living animals.

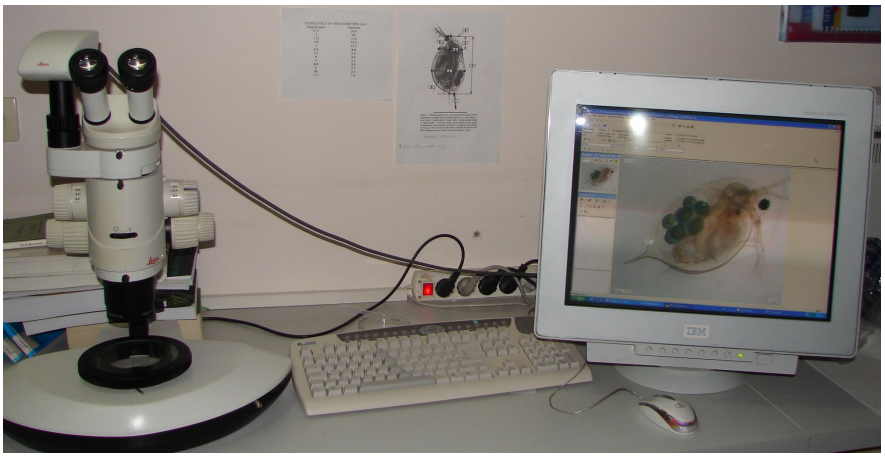

Figure 2.9 Leica M Stereo Microscope–MZ16 and attached DFC280 camera system (METU Limnology Laboratory).

- head length of the animal (1)
- body length of the animal (2)
- body width of the animal (3)
- egg size of the animal (4)
- egg number of the animal
- age at first reproduction
- size at first reproduction
- neonate size of the animal
- neonate number of the animal

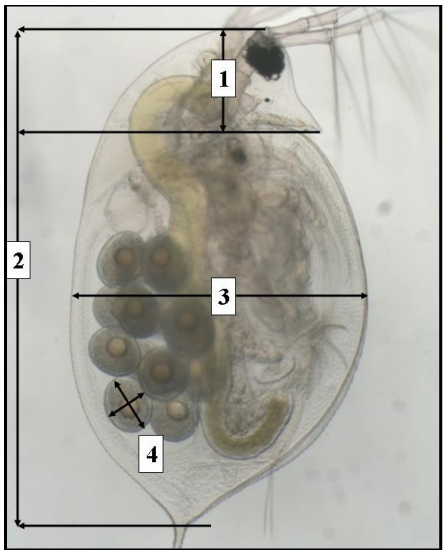

Figure 2.10 Morphometric characters of *Daphnia pulex* (Photo was taken by Gizem Bezirci 2007 at METU Limnology Lab).

2.5 Statistical Models

Three statistical models were use to evaluate the data:

SAS-GLM Repeated Measures of 2-Way ANOVA (SAS Version 9.00)

This test was used to determine both single and multistressor effects in a certain time. Survival and body length (body length, body width) data were examined.

EPA Probit Analysis Program (Version 1.5)

This test was used to determine 24 and 48 hours LC (Lethal Concentration) in the acute toxicity experiments. Using LC_{50} (concentration estimated to kill 50 per cent of the population) values, the salinity levels of the chronic experiments were determined.

Dunnett's Pairwise Multiple Comparison t-test (SPSS 13.0 for Windows)

This test was used for pairwise comparison of survival ratio, body lengths (body length, body width), egg number, age at first reproduction, size at first reproduction and neonate number.

CHAPTER 3

RESULTS

To investigate the effects of multistressors, which included salinity, temperature, food and fish predation pressure on the survival and life history traits of *Daphnia pulex*, several acute and chronic toxicity experiments were performed. Results of the experiments were given below in the order of first acute toxcity experiments and then the chronic toxicity experiments.

3.1 Acute Toxicity Experiments

A) Acute Toxicity Experiment Using High Doses of Salinity

As there has not been any investigation to study the separate and the combined effects of salinity and fish-exuded kairomone on survival of *D.pulex*, thus the first acute experiment was designed to determine the resistance of *Daphnia pulex* to the highest salinity levels and following results were obtained.

Figure 3.1-A&B shows the percent survival in the absence/presense of fish-exuded kairomone (F–) and (F+), respectively while Table 3.1 presents the results of repeated measuresof 2-way ANOVA and Table 3.2 presents the results of Dunnet's pairwise comparison applied to the survival data of the *D. pulex* individuals over time during a acute exposure to salinity. It was observed that salinity, fish-exuded kairomone and time had significant impacts on the survival of daphnids ($p < 0.001^{***}$, Table 3.1, Figure 3.1- B). Up to 2 g/L salinity dose, presence of fish-exuded kairomone affected

survival in a positive way but along with the increasing salinity, this positive effect disappeared and in both absence (F-) and presence (F+) of fish-exuded kairomone, survival of the daphnids decreased significanlty, thus begginning from the third salinity dose (4.00 g/L), all of the daphnids were dead in 24 hours time (Table 3.2, Figure 3.1- A&B).

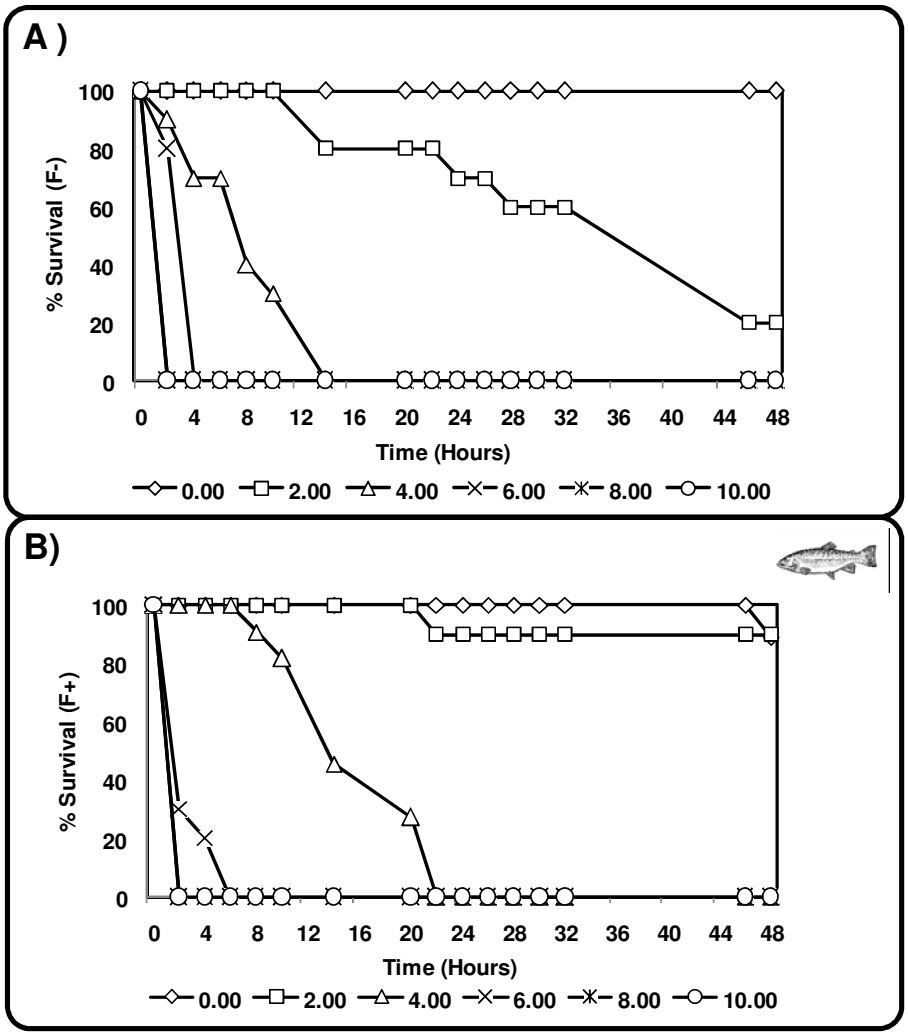

Figure 3.1. Percent survival of the *D. pulex* individuals over 48 hours acute exposure to salinity in the absence (F–) (A-upper graph) and presence (F+) (B-below graph) of fish-exuded kairomone.

Table 3.1. Results of repeated measures of 2-way ANOVA applied to the survival data of *D. pulex* over time during an acute exposure to salinity.

	Treatment	p-value
Impact	**Fish**	0.0002***
	Salinity	<.0001***
	Time	<.0001***
Interaction	**Fish*Salinity**	0.0007***
	Fish*Time	0.0580
	Salinity*Time	<.0001***
	Fish*Time*Salinity	<.0001***

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.2. Dunnett's pairwise comparison applied to the survival data of *D. pulex* over time during an acute high-dose exposure to salinity in the absence (F–) and presence (F+) of fish-exuded kairomone.

Treatment	F- 0 g/L	F+ 0 g/L
2 g/L	.000***↓	0.492
4 g/L	.000***↓	.000***↓
6 g/L	.000***↓	.000***↓
8 g/L	.000***↓	.000***↓
10 g/L	.000***↓	.000***↓

**** P < 0.001, ** P < 0.01, * P < 0.05*

Recorded data couldn't be evaluated for probit analyses because the survival of the individuals were so low but they were used to design second acute toxicity experiment which included lower doses compared to this acute one.

B) Second Acute Toxicity Experiment

While planning this acute toxicity experiment, results of the previous experiment were examined and thus lower doses of salinity were choosen compared to the first one. Figure 3.2-A&B shows the percent survival in the absence/presense of fish-exuded kairomone (F–) and (F+), while Table 3.3 present the results of repated measures of 2-way ANOVA and Table 3.4 present the results of Dunnet's pairwise comparison applied to the survival data of the *D. pulex* individuals over time during a acute exposure to salinity. It was observed that survival of the animals were affected significantly with salinity, presence of fish-exuded kairomone and time ($p < 0.001^{***}$, Table 3.3). In two highest salinity levels (5.00 and 6.50 g/L) , survival of the animals were decreased significantly both in the absence (F-) and presence (F+) of fish-exuded kairomone. In those two doses, most of the animals were dead in 48 hours ($p < 0.001^{***}$, Table 3.4, Figure 3.2-A&B) . But in the presence (F+) of fish-exuded kairomone, *D. pulex* individuals were tend to be more resistant to salinity. When we compare both 24 and 48 hours of LD_{50} results it was found that in the presence (F+) of fish-exuded kairomone, LD_{50} values are higher than absence(F-) of fish-exuded kairomone.

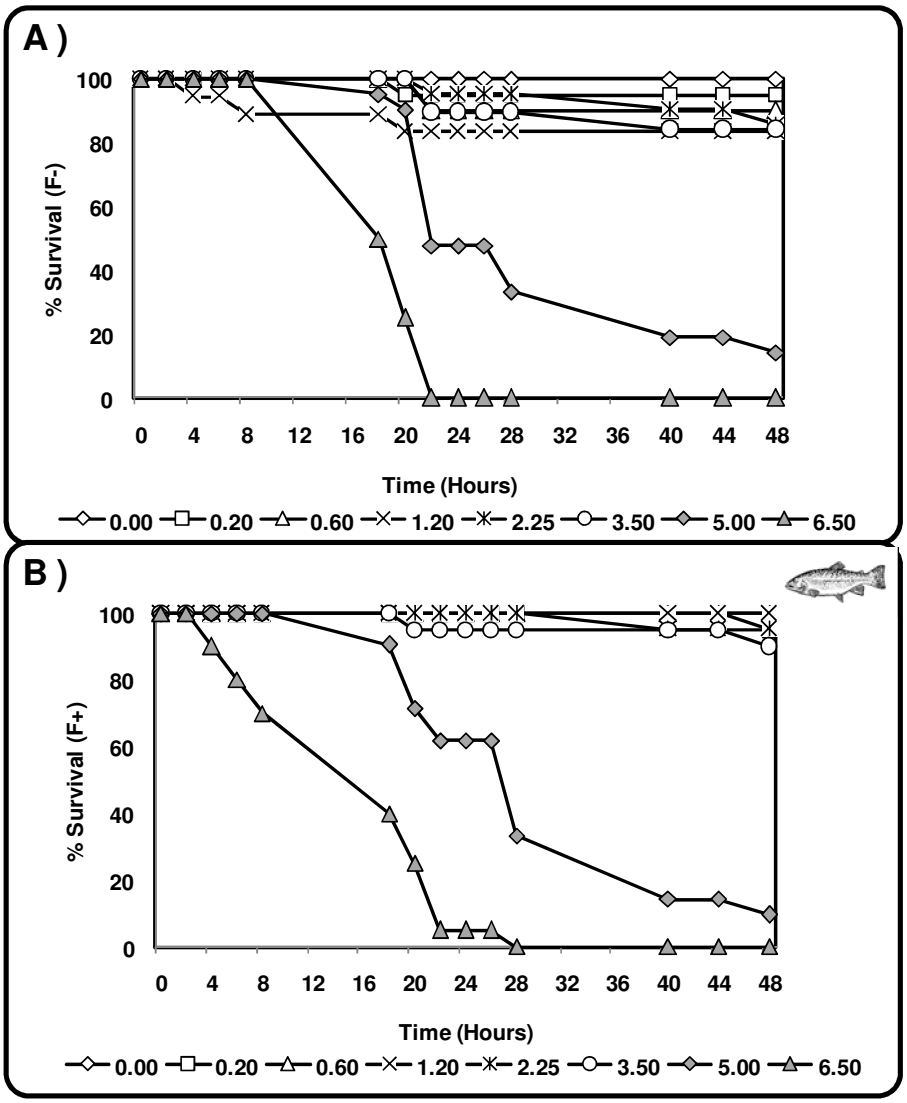

Figure 3.2. Percent survival of the *D. pulex* individuals over 48 hours acute exposure to salinity in the absence (F–) (A-upper graph) and presence (F+) (B-below graph) of fish-exuded kairomone.

Table 3.3. Results of repeated measures of 2-way ANOVA applied to the survival data of *D. pulex* over time during an acute exposure to salinity.

	Treatment	p-value
Impact	**Fish**	0.0066***
	Salinity	<.0001***
	Time	<.0001***
Interaction	**Fish*Salinity**	<.0001***
	Fish*Time	0.3242
	Salinity*Time	<.0001***
	Fish*Time*Salinity	1.0000

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.4. Dunnett's pairwise comparison applied to the survival data of *D. pulex* over time during an acute high-dose exposure to salinity in the absence (F–) and presence (F+) of fish-exuded kairomone.

Threatment	F- 0.00 g/L	F+ 0.00 g/L
0.20 g/L	.994	1.000
0.60 g/L	.864	1.000
1.20 g/L	.155	1.000
2.25 g/L	.919	1.000
3.50 g/L	.282	.861
5.00 g/L	.000***↓	.000***↓
6.00 g/L	.000***↓	.000***↓

**** P < 0.001, ** P < 0.01, * P < 0.05*

Than LD_{50} values of salinity in the absence and presence of fish-exuded kairomone were calculated using probit analyses (EPA Probit Analysis Program Version 1.5) as above:

In the absence of fish-exuded kairomone: **24-h LD_{50}** : 4.754 g/L **48-hLD_{50}**:3.260 g/L

In the presence of fish-exuded kairomone: **24-h LD_{50}** : 5.040 g/L **48-hLD_{50}**: 3.774 g/L

C) Third Acute Toxicity Experiment Using Low Doses of Salinity

Both of the previous high dose-acute toxicity experiments were used to select the new doses used in this experiment.

Figure 3.3-A&B shows the percent survival in the absence/presense of fish-exuded kairomone (F–) and (F+), while Table 3.5 present the results of rm-2-way ANOVA and Table 3.6 present the results of Dunnett's pairwise comparison applied to the survival data of the *D. pulex* individuals over time during an acute exposure to salinity. It was found that both salinity, fish-exuded kairomone and time had significant effets on the survival ($p < 0.001^{***}$, Table 3.4). In addition to that, different interactions of salinity, fish and time had all significant impacts on the survival of the daphnids except salinity-fish interaction ($p < 0.001^{***}$, Table 3.4). Presence of fish predation significantly reduced the effect of salinity especially at the low and intermediate doses of salinity. In the absence of fish- exuded kairomone (F-), survival of the animals decreased starting from the third dose (0.25 g/L) of the salinity levels, but in the presence of fish-exuded kairomone (F+) survival decreased significantly only in two high salinity doses (2.50 and 5.00 g/L) (Figure 3.3- A&B, Table 3.5).

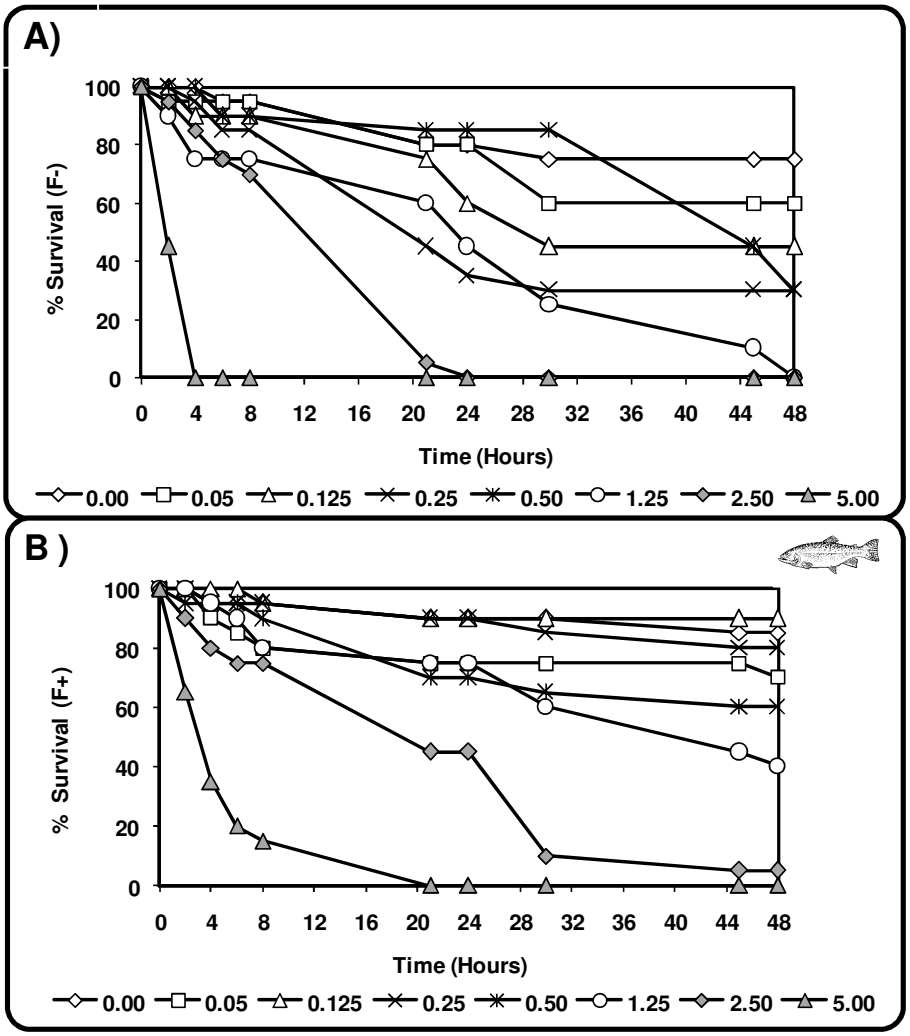

Figure 3.3. Percent survival of the *D. pulex* individuals over 48 hours acute exposure to salinity in the absence (F–) (A-upper graph) and presence (F+) (B-below graph) of fish-exuded kairomone.

Table 3.5. Results of repeated measures of 2-way ANOVA applied to the survival data of *D. pulex* over time during an acute exposure to salinity.

	Treatment	p-value
Impact	**Fish**	<.0001***
	Salinity	<.0001***
	Time	<.0001***
Interaction	**Fish*Salinity**	0.0599
	Fish*Time	<.0001***
	Salinity*Time	<.0001***
	Time*Fish*Salnity	<.0001***

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.6. Results of Dunnett's pairwise comparison applied to the survival data of *D. pulex* over time during an acute exposure to salinity in the absence (F–) and presence (F+) of fish-exuded kairomone.

Treatment	F–	F+
	0.00 µg/L	**0.00 µg/L**
0.05 g/L	.972	.561
0.125 g/L	.210	1.000
0.25 g/L	.009**↓	1.000
0.50 g/L	-	.348
1.25 g/L	.001***↓	.174
2.50 g/L	.000***↓	.000***↓
5.00 g/L	.000**↓	.000***↓

**** P < 0.001, ** P < 0.01, * P < 0.05*

The LD_{50} for salinity in the absence and presence of fish-exuded kairomone were determined using probit analyses (EPA Probit Analysis Program Version 1.5) as above. They were;

In the absence of fish-exuded kairomone: **24-h LD_{50}** :0.401 g/L **48-hLD_{50}** : 0.159 g/L

In the presence of fish-exuded kairomone: **24-h LD_{50}** :1.962 g/L **48-h LD_{50}** : 1.007 g/L

In the presence of fish predation pressure the lethal doses of salt that kill the half of the daphnids in 24 and 48 hrs were five and six times higher, respectively.

3.2 Chronic Toxicity Experiments

3.2.1 Survival Experiments

A) Effect of Salinity and Fish Predation on The Survival of *Daphnia pulex*

Effects of salinity and fish predation on the survival of *Daphnia pulex* were investigated by chronic toxicity experiment.

Figure 3.4-A&B shows the percent survival in the absence/presense of fish-exuded kairomone (F–) and (F+), while Table 3.7 presents the results of rm-2-way ANOVA and Table 3.8 presents the results of Dunnet's pairwise comparison applied to the survival data of the *D. pulex* individuals over time during a chronic exposure to salinity and fish-exuded kairomone. Salinity and time both had significant effects on the survival of daphnids either alone or in a combination whereas fish predation had no significant effect though predation effect showed the tendency of increasing survival of the daphids (Table 3.7). In the absence (F-) of fish-exuded kairomone, survival rates of the first three doses (0.05, 0.10, 0.20 g/L salt) were similar to the control group and in the presence (F+) of fish-exuded kairomone, survival rates of the first four doses (0.05, 0.10, 0.20, 0.40 g/L salt) were also similar to the control group (Table 3.8). Survival of the individuals decreased significanlty with the increasing salinity especially in two of the high doses (0.80 and 1.50 g/L) in both absence (F-) and presence (F+) of fish-exuded kairomone (Table 3.8, Figure 3.4-A&B).

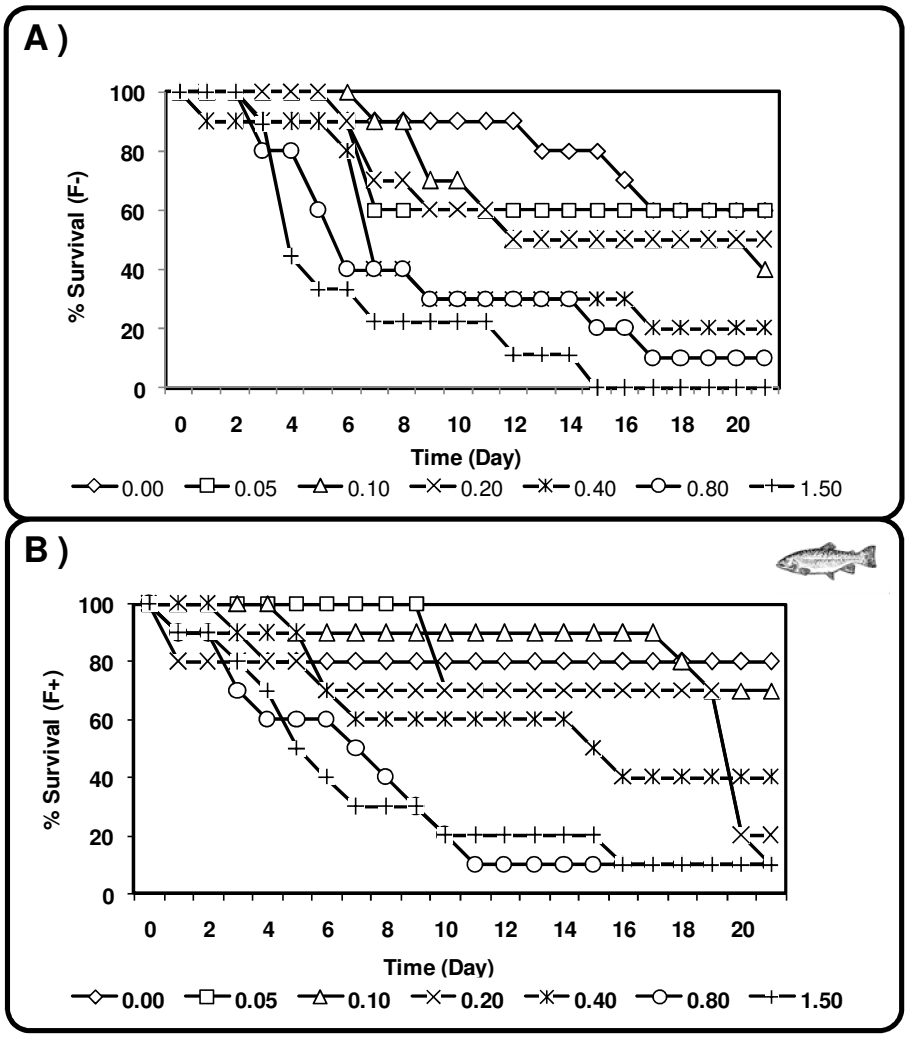

Figure 3.4. Percent survival of the *D. pulex* individuals over 21 days chronic exposure to salinity in the absence (F–) (A-upper graph) and presence (F+) (B-below graph) of fish-exuded kairomone.

Table 3.7. Results of repeated measures of 2-way ANOVA applied to the survival data of *D. pulex* over time during an chronic exposure to salinity.

	Treatment	p-value
Impact	**Fish**	0.8796
	Salinity	<.0001***
	Time	<.0001***
Interaction	**Fish*Salinity**	0.7710
	Fish*Time	0.4236
	Salinity*Time	<.0001***
	Fish*Salinity*Time	<.0001***

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.8. Results of Dunnett's pairwise comparison applied to survival data of *D. pulex* over time during a chronic exposure to salinity in the absence (F–) and presence (F+) of fish-exuded kairomone.

Threatment	F- 0.00 g/L	F+ 0.00 g/L
0.05 g/L	.829	1.000
0.10 g/L	.761	1.000
0.20 g/L	.759	.931
0.40 g/L	.051↓	.791
0.80 g/L	.015*↓	.010**↓
1.50 g/L	.003**↓	.002**↓

**** P < 0.001, ** P < 0.01, * P < 0.05*

A) Effects of Multitressors on The Survival of *Daphnia pulex*

The interaction effects of multistressors including temperature, salinity, food and fish-exuded kairomone on the survival of *D. pulex* was investigated by a chronic toxicity experiment.

Percent survival of the *D. pulex* individuals over time during chronic exposure to salinity, fish-exuded kairomone and different food levels in high temperature (Figure 3.5-A) and in low temperature (Figure 3.5-B) were presented. The mean day of death of *D. pulex* individuals in absence (F-) and presence (F+) of fish-exuded kairomone was shown in Figure 3.6-A&B. The results of rm-2-way ANOVA and Dunnet's pairwise comparison applied

to the survival data of the *D. pulex* individuals over time during a chronic exposure to temperature, salinity, food and fish-exuded kairomone were shown in Table 3.9 and Table 3.10.

Except from food, other stressors (temparature, salinity, fish-exuded kairomone, time) had significant impacts on the survival of daphnids. As considered the multiple stressors including combinations of fish-exuded kairomone with salinity, time and temperature had also significant effects on the survival of *D. pulex* individuals. As we compared triple interactions, it was found that, combination of fish-exuded kairomone, salinity and temperature through time had significant impacts on the survival (Table 3.8).

Table 3.9. Results of repeated measures of 2-way ANOVA applied to the survival data of *D. pulex* over time during an chronic exposure to salinity, fish-exuded kairomone, temperature and food.

	Treatment	p-value
Impact	Fish	0.0003***
	Salinity	<.0001***
	Temperature	0.0002***
	Food	0.3004
	Time	<.0001***
2-way Interactions	Fish*Salinity	0.0032**
	Fish*Temperature	<.0001***
	Fish*Food	0.3536
	Salinity*Temperature	0.4389
	Salinity*Food	0.2065
	Temperature*Food	0.9497
	Time*Fish	<.0001***
	Time*Salinity	<.0001***
	Time*Temperature	<.0001***
	Time*Food	0.6164
3-way Interactions	Time*Fish*Salinity	0.0005***
	Time*Fish*Temperature	<.0001***
	Time*Fish*Food	0.2934
	Time*Salinity*Temperature	0.1268
	Time*Salinity*Food	<.0001***
	Time*Temperature*Food	0.1475

**** P < 0.001, ** P < 0.01, * P < 0.05*

The combination of regular temperature, optimum and low food conditions, fish-exuded kairomone and in all salinity doses, survival of the animals decreased though in the presence of fish-exuded kairomone (F+), decrease in the survival was more clear when it was compared to the absence (F-) of it (Table 3.9, Figure 3.5-A). In addition to that, the highest salinity dose (1.50 g/L) significantly decreased the survival (Table 3.10). Although the effect of food was not significant in two-way ANOVA test, in the optimum food condition along with fish-exuded kairomone in regular temperature (22±1 °C) the survival was much lower. In all treatments, exposure to the low food availability, survival of the daphnids remianed significantly high compared to the optimum food conditions.

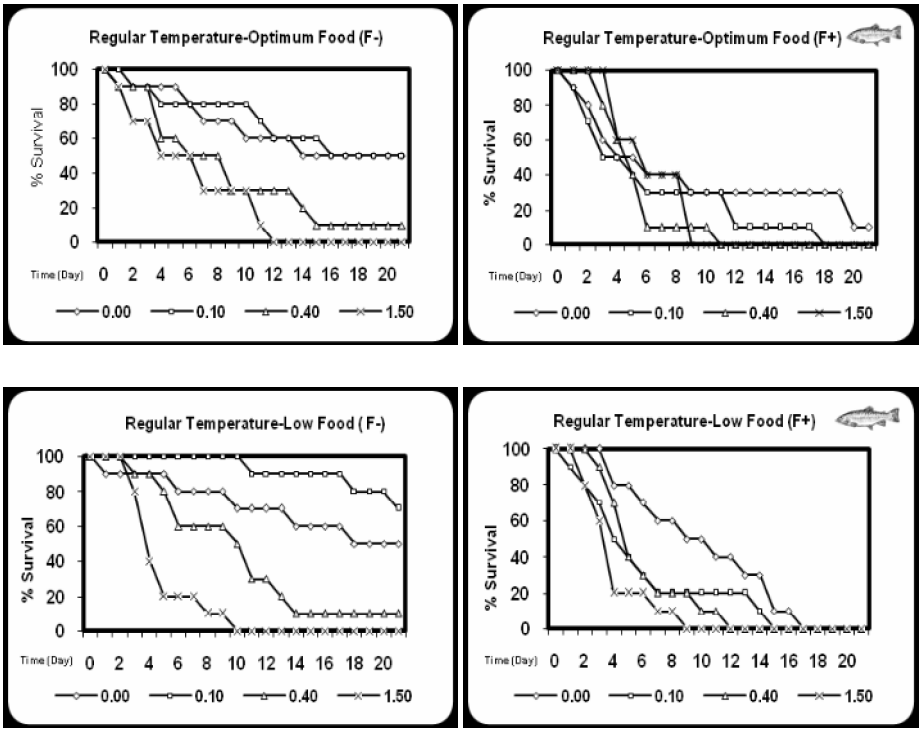

Figure 3.5-A. Percent survival of the *D. pulex* individuals over time during chronic exposure to salinity, fish-exuded kairomone and different food levels at regular temperature (22±1 °C). While left column represent absence of fish-exuded kairomone (F-), right column represent presence of fish-exuded kairomone (F+).

High temperature significantly reduced the survival compared to the regular temperature. The effect of temperature was enhanced with inclusion of food effect when given at optimum level, further reduced the survival. At high temperature treatments, contraray to the regular temperature, fish-exuded kairomone did not come up strong on survival of the daphnids (Table 3.9).

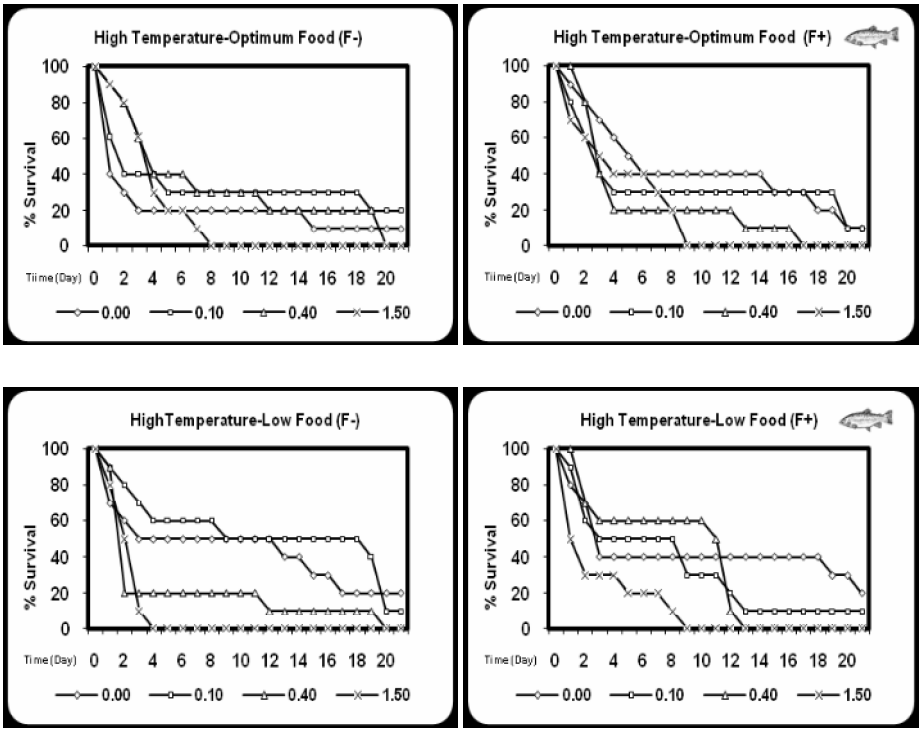

Figure 3.5-B. Percent survival of the *D. pulex* individuals over time during chronic exposure to salinity, fish-exuded kairomone and different food levels at high temperature (25±1 °C). While left column represent absence of fish-exuded kairomone (F-), right column represent presence of fish-exuded kairomone (F+).

Table 3.10. Results of Dunnett's pairwise comparison applied to survival data of *D. pulex* over time during chronic exposure to salinity, two different temperatures (regular and high), two different food levels (optimum and low), in the absence (F–) and presence (F+) of fish-exuded kairomone.

0.00 g/L								
	Regular Temperature				High Temperature			
	Optimum Food		Low Food		Optimum Food		Low Food	
Thereatment	**F-**	**F+**	**F-**	**F+**	**F-**	**F+**	**F-**	**F+**
0.10 g/L	.991	.585	.146	.061	.713	.877	.782	.788
0.40 g/L	.155	.304	.052	.061	.671	.386	.296	.942
1.50 g/L	.022*↓	.585	.000***↓	.003**↓	.995	.187	.072↓	.063↓

**** P < 0.001, ** P < 0.01, * P < 0.05*

In the absence of fish-exuded kairomone, for regular temperature (RT) the mean day of death was significantly higher than high temperature (HT). When food effect was included, in the optimum food (OF) treatments, the mean day death was higher than low food (LF) treatments. (Figure 3.6-A). In the presence of fish for both temperature and food treatments, the mean day death became much lower. However, the effects of the high and low temperature and food levels showed the same trend of responses as found in the absence of fish-exuded kairomone. (Figure 3.6-B). Daphnids benefited from a slight increase in salinity, however, further increase led to significant decrease in survival ($p < 0.001^{***}$), though salinity effect was more pronounced in the fishless treatments. A small increase in salinity (0.1‰), there was a minor but positive effect (lowered mortality), while with a further increase (0.4 ‰ and more) mortality rises rapidly.

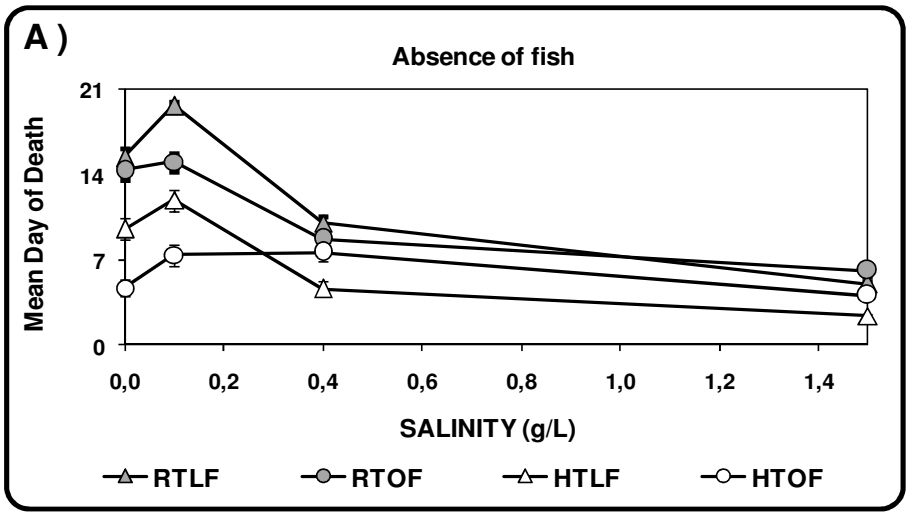

Figure 3.6-A. Mean day of death of *D. pulex* individuals in absence of fish-exuded kairomone (F-) . RTLF: Regular temperature-Low food, RTOF: Regular temperature-Optimum food, HTLF:High temperature-Low food, HTOF:High temperature-Optimum food.

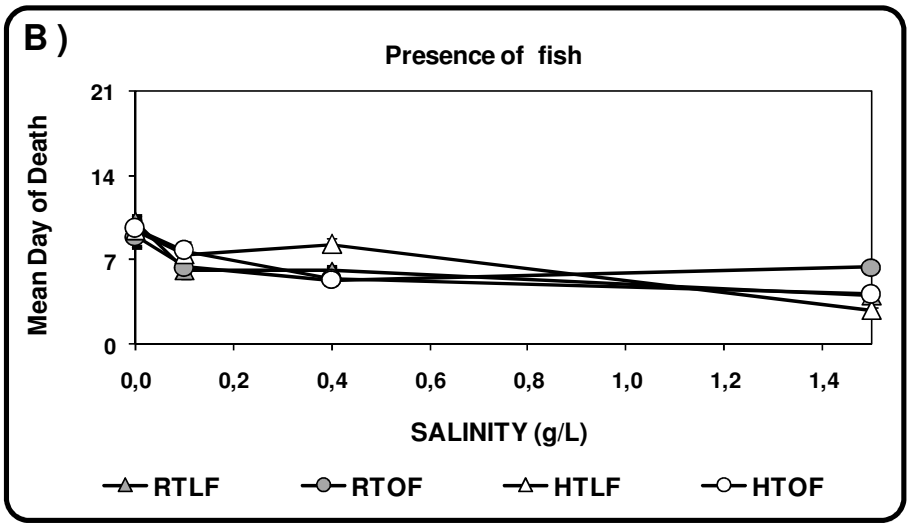

Figure 3.6-B. Mean day of death of *D. pulex* individuals in the presence of fish-exuded kairomone (F+). RTLF: Regular temperature-Low food, RTOF: Regular temperature-Optimum food, HTLF:High temperature-Low food, HTOF:High temperature-Optimum food.

3.2.2 Life History Traits Experiment

A) Influence of Salinity and Fish Predation on Life History Traits of *Daphnia pulex*

Combined effects of salinity and fish-exuded kairomone on the survival and life history traits of *Daphnia pulex* were investigated in a separete chronic toxicity experiments.

Figure 3.7-A&B shows the percent survival in the absence of fish-exuded kairomone (F–) and Figure 3.14 shows percent survival in the presence of fish-exuded kairomone (F+), while Table 3.11 present the results of rm-2-way ANOVA applied to the survival data of the *D. pulex* individuals over time during a chronic exposure to salinity. It was observed that salinity decreased survival significantly through the course of the experiment (Table 3.11) hence in the two high salinity doses (0.8 and 1.2 g/L), most of the indivudals died eight days after the start of the experiment. This could imply that longer exposure of the toxicant could be more dreadful for the animals. Presence of fish kairomone (F+) seems to had no significant effect of survival alone but when it was considered together with salinity, they had significant (Table 3.10) effect on the survival of daphnids.

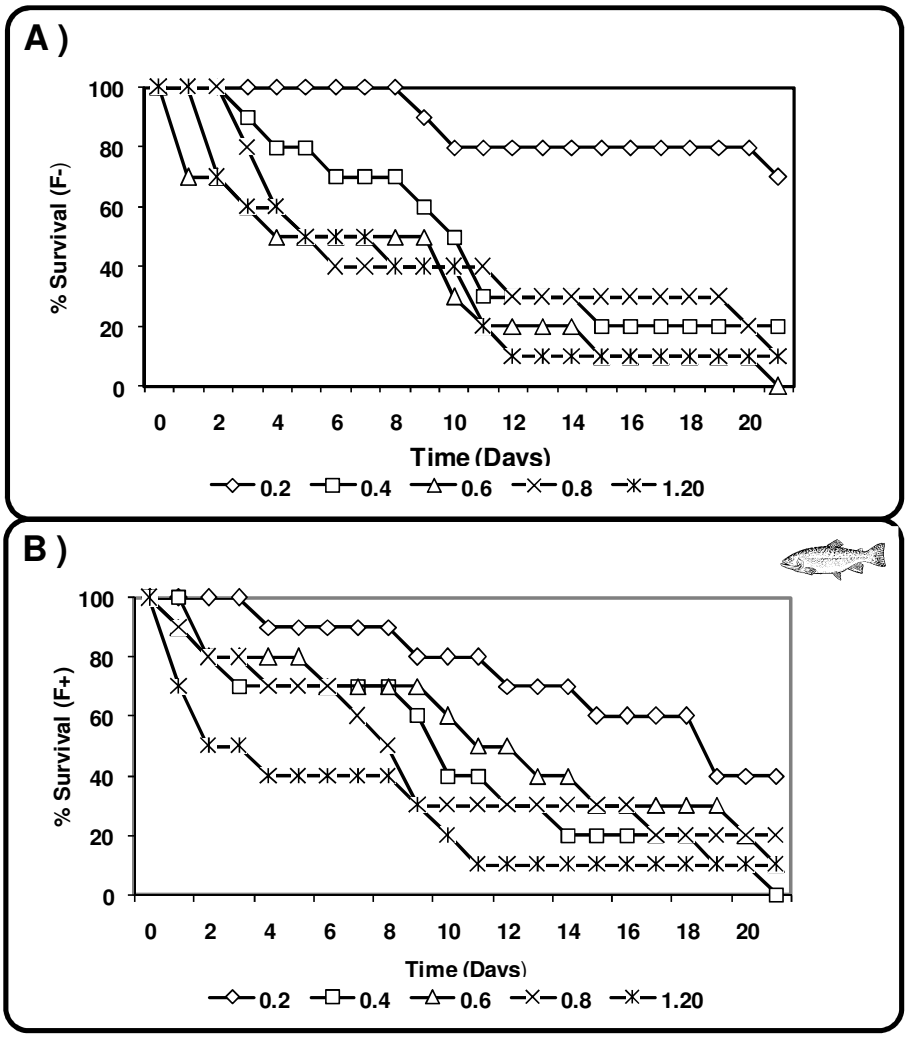

Figure 3.7. Percent survival of the *D. pulex* individuals over 21 days chronic exposure to salinity in the absence (F–) (A-upper graph) and presence (F+) (B-below graph) of fish-exuded kairomone.

Table 3.11. Results of repeated measures of 2-way ANOVA applied to the survival data of *D. pulex* over time during an chronic exposure to salinity.

Treatment	p-value
Fish	0.3999
Salinity	<.0001***
Time	<.0001***
Fish*Salinity	<.0001***
Salinity*Time	0.9853
Fish*Time	0.9999

**** P < 0.001, ** P < 0.01, * P < 0.05*

Figure 3.8 presents the average egg number, while Table 3.12 and Table 3.13 present the results of rm-2-way ANOVA and Dunnett's pairwise comparison applied to the egg number data of *D. pulex* individuals over time during the chronic exposure to salinity in the absence (F–) and presence of fish-exuded kairomone (F+). Table 3.12 showed that salinity significantly decreased the the egg numbers (clutch size). Figure 3.8 and Table 3.13 revealed that the average egg number decreased significantly especially at 0.60, 0.80 and 1.20 g/L salinity doses and in the presence of fish-exuded kairomone (F+) number of eggs increased when compared to the abscence of fish treatment (F–).

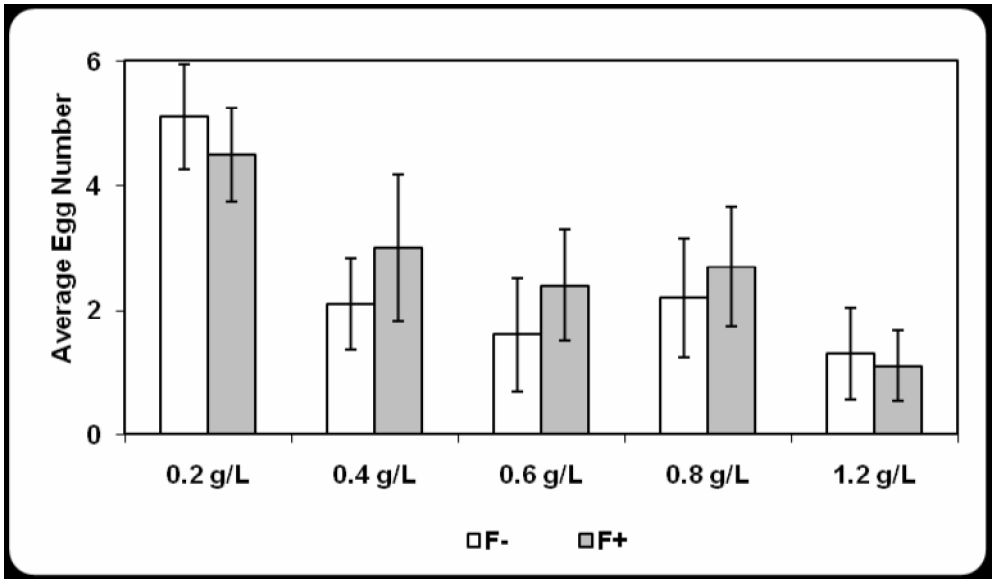

Figure 3.8. Average egg number of *D. pulex* individuals during a chronic exposure to salinity in the absence (open bars) and presence (filled bars) of fish-exuded kairomone (Mean±Std Error).

Table 3.12. Results of repeated measures of 2-way ANOVA applied to the egg number data of *D. pulex* over time during an chronic exposure to salinity.

Treatment	Egg Number
Fish	0.612
Salinity	0.002**
Fish*Salinity	0.889

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.13. Results of Dunnett's pairwise comparison applied to egg number data of *D. pulex* over time during a chronic exposure to salinity in the absence (F–) and presence (F+) of fish-exuded kairomone.

0.2 g/L		
Egg Number		
Treatment	**F-**	**F+**
0.40 g/L	0.052	0.583
0.60 g/L	0.018*↓	0.293
0.80 g/L	0.063↓	0.425
1.20 g/L	0.009**↓	0.034*↓

**** P < 0.001, ** P < 0.01, * P < 0.05*

Figure 3.9 represents age at first reproduction and Figure 3.17 represents size at first reproduction, while Table 3.14 and 3.15 present the results of rm-2-way ANOVA and Dunnett's pairwise comparison applied to the age and size at first reproduction data, respectively of *D. pulex* individuals over time during the chronic exposure to salinity in the absence (F–) and presence of fish-exuded kairomone (F+). It was observed that, age at first reproduction wasn't affected by salinity significantly but in the presence of fish-exuded kairomone (F+), it was tend to decreas (Table 3.15, Figure 3.9). Except from the highest salinity dose (1.2 g/L), age at first reproduction was decreased in the presence of fish-exuded kairomone (Table 3.15, Figure 3.9). Size at first reproduction also wasn't significantly affected with salinty but in the presence of fish-exuded kairomone (F+), size at first reproduction decreased significantly (Table 3.14). As it was shown in Figure 3.10, the size at first reproduction decreased in the presence of fish-

exuded kairomone when compared to the absence of fsih-exuded kairomone data.

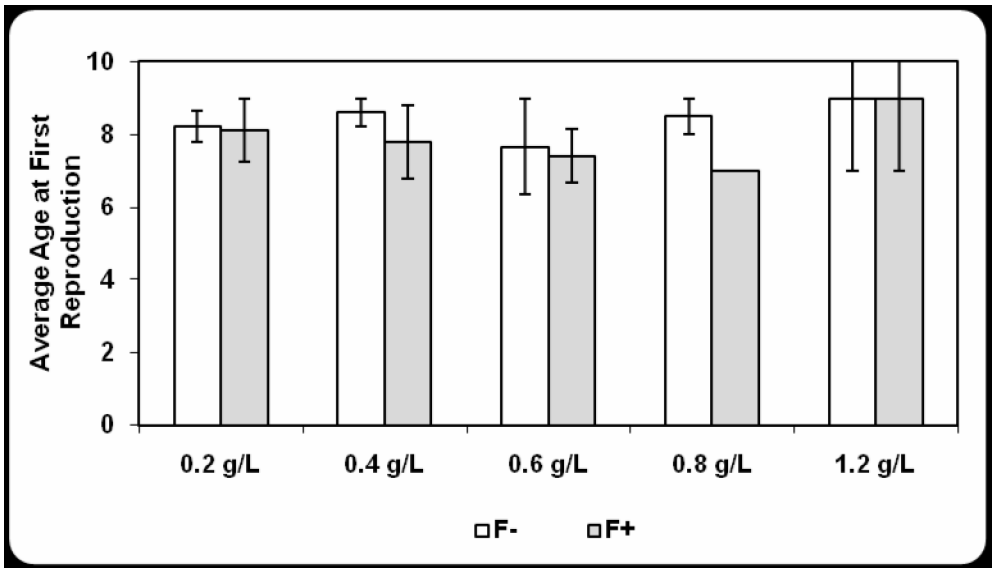

Figure 3.9 Age at first reproduction of *D. pulex* individuals during a chronic exposure to salinity in the absence (open bars) and presence (filled bars) of fish-exuded kairomone (Mean±Std Error).

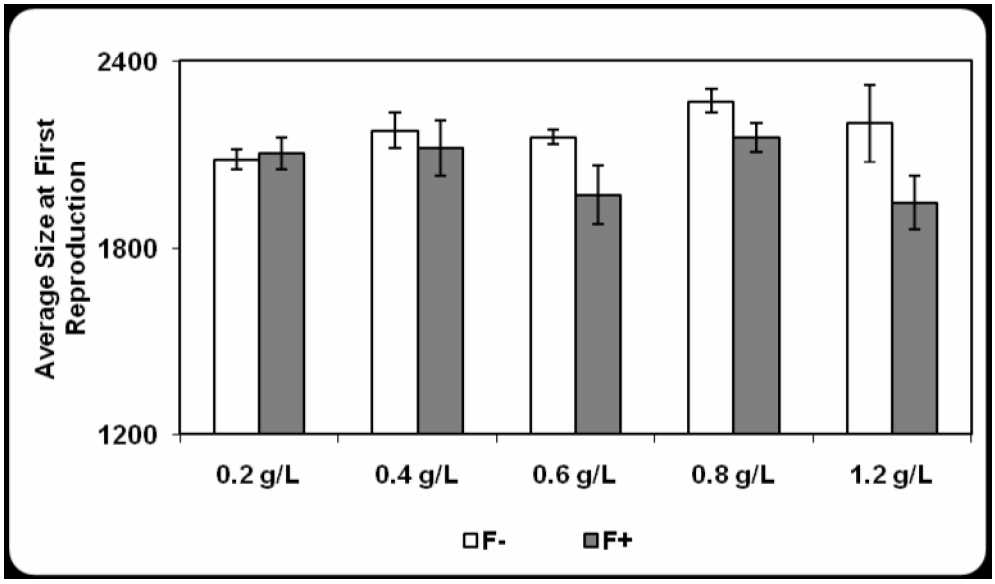

Figure 3.10 Size at first reproduction of *D. pulex* individuals during a chronic exposure to salinity in the absence (open bars) and presence (filled bars) of fish-exuded kairomone (Mean±Std Error).

Table 3.14. Results of repeated measures of 2-way ANOVA applied to the age at firts reproduction and size at first reproduction data of *D. pulex* over time during an chronic exposure to salinity.

Treatment	Age at First Reproduction	Size at First Reproduction
Fish	0.397	0.012*
Salinity	0.752	0.217
Fish*Salinity	0.926	0.289

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.15. Results of Dunnett's pairwise comparison applied to age at first reproduction and size at first reproduction data of *D. pulex* over time during a chronic exposure to salinity in the absence (F–) and presence (F+) of fish-exuded kairomone.

0.2 g/L				
	Age at First Reproduction		Size at First Reproduction	
Treatment	**F-**	**F+**	**F-**	**F+**
0.40 g/L	0.976	0.998	0.402	0.999
0.60 g/L	0.965	0.957	0.761	0.359
0.80 g/L	0.994	0.860	0.032*↑	0.950
1.20 g/L	0.921	0.132	0.514	0.481

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.16 presents the results of repeated measures of 2-way ANOVA applied to the body length and body width data of *D. pulex* over time during chronic exposure to salinity, in the presence (F+) and absence (F-) of fish-exuded kairomone. Figure 3.11 represents the body length/body width ratio, while Table 3.17 and 3.18 present the results of rm-2-way ANOVA and Dunnett's pairwise comparison applied to the body length/body width ratio data of *D. pulex* individuals over time during the chronic exposure to salinity in the absence (F–) and presence of fish-exuded kairomone (F+). While fish-exuded kairomone and salinity had significant impacts on the body length with time, body width was controlled by all stressors significantly (Table 3.16). In addition to that, body length/body width ratio was significatly affected by salinity and fish-exuded kairomone (Table

3.17,Figure 3.11). Table 3.17 showed that both salinity ($p < 0.001^{***}$) and fish-exuded kairomone ($p < 0.05^{*}$) had significant effects on the body length/body width data. Figure 3.11 and Table 3.18 revealed that body length/body width ratio decreased significantly ($p < 0.001^{***}$) in the abscence of fish-exuded kairomone (F–) at 0.60, 0.80 and 1.20 g/L salinity doses. In the presence of fish-exuded kairomone (F+) only significant difference was observed at 0.80 g/L salinity dose ($p < 0.001^{***}$).

Table 3.16. Results of repeated measures of 2-way ANOVA applied to the body length and body width data of *D. pulex* over time during an chronic exposure to salinity.

	Treatment	Body Length	Body Width
Impact	**Fish**	0.7292	<.0001***
	Salinity	0.0787	<.0001***
	Time	<.0001***	<.0001***
Interaction	**Fish*Salinity**	0.5045	<.0001***
	Time*Fish	0.0018**	<.0001***
	Time*Salinity	<.0001***	<.0001***
	Time*Fish*Salinity	0.6569	<.0001***

**** P < 0.001, ** P < 0.01, * P < 0.05*

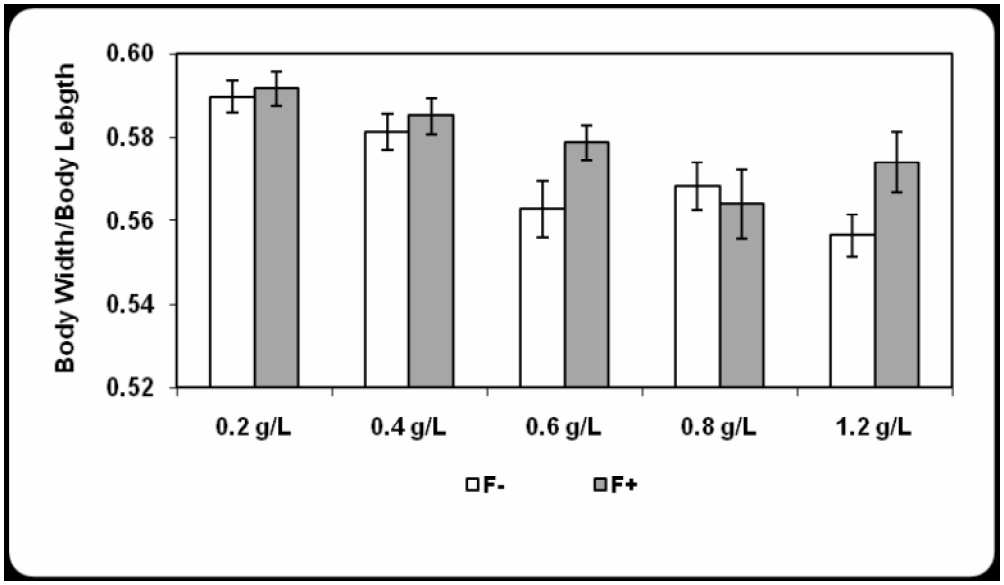

Figure 3.11. Body length / Body width ratio of *D. pulex* individuals during a chronic exposure to salinity in the absence (open bars) and presence (filled bars) of fish-exuded kairomone (Mean±Std Error).

Table 3.17. Results of repeated measures of 2-way ANOVA applied to the body length / body width data of *D. pulex* over time during an chronic exposure to salinity.

Treatment	Body Length/Body Width
Fish	0.048*
Salinity	0.000***
Fish*Salinity	0.293

**** P < 0.001, ** P < 0.01, * P < 0.05*

Table 3.18. Results of Dunnett's pairwise comparison applied to the body length / body width data of *D. pulex* over time during a chronic exposure to salinity in the absence (F–) and presence (F+) of fish-exuded kairomone.

	0.20 g/L	
	Body Length/Body Width	
Treatment	**F-**	**F+**
0.40 g/L	0.514	0.751
0.60 g/L	0.001***↓	0.182
0.80 g/L	0.006**↓	0.001***↓
1.20 g/L	0.000***↓	0.120

**** P < 0.001, ** P < 0.01, * P < 0.05*

CHAPTER 4

DISCUSSION

Daphnia is one of the most important components of freshwater planktonic communities. It is a significant herbivore that affects the composition and abundance of algal communities and is also a significant prey item for invertebrate predators (Larson and Dodson, 1993). Phenotypic plasticity (Cousyn *et al*., 2001) and flexible age-specific reproductive output and survival, collectively, form a greater part of life history strategies of Cladocera (Innes and Singleton, 2000), which facilitate their successful colonization in diverse habitats (Sarma *et al*., 2005).

Combating stress is likely to be energetically costly for stressed organisms (Calow, 1991; Calowand and Sibly, 1990; Genoni, 1997; De Coen and Janssen, 2003). If, indeed, stress will increase the energy expenditure of organisms, the energy status of an organism at any given time should affect its capacity to cope with stress, too (Smolders *et al*., 2005).

Organisms can be exposed to a mixture of different toxicants in the environment and although the toxicity of the single compounds might be well known, their simultaneous presence might induce non-overlapping toxic effects (Goldoni and Johansson, 2007). Natural stressors such as, temperature, salinity, predation pressure, food availability have strong control on daphnids survival and reproduction (Lampert, 1985; Urabe *et al*., 1997; Ferrao-Filho and Azevedo, 2003; Von Elert, 2002; Lynch, 1978; DeMott, 1983; Hu and Tessier, 1995).

Globally, freshwater ecosystems are showing changes in organism abundance and productivity, range expansions, and phenological shifts

(including earlier fish migrations) that are linked to rising temperatures (IPCC 2007). Strecker *et al*. (2004) showed that moderate warming could change the community composition and reduce the availability of food for higher tropic levels. Beisner *et al*. (1997) found that predator–prey equilibrium could be rapidly destabilized with an increase in temperature in a system with three tropic levels, including an invertebrate predator, which was dynamically coupled to herbivore. Those changes and interactions between populations are crucial to the estimation of the impact of global warming on aquatic food webs (Wagner and Benndorf, 2007). And timing of interactions between populations and the resulting consequences for population dynamics and ecosystem processes have emerged as central mechanisms of how global change will modify the biotic structures of ecosystems (Harrington *et al*., 1999; Visser and Both, 2005).

Daphnia, a keystone species for freshwater ecosystems, not only playing a major role in the freshwater foodweb but also they are controlling the growth of algal communities and as a result of that they enhance the water clarity. Changes in the environmental conditions could effects both the survival and life cycle of the animals differently. Natural changes, such as the seasonal evaporation of water from freshwater bodies, can contribute to an increase in the salinity of many aquatic ecosystems (Das *et al*., 1995; Williams *et al*., 1998). As a result of global warming, decrease in the surface runoff waters and increased evaporation can enhance salinisation in freshwaters (Wetzel, 1983; Jeppesen *et al*., 1994; Beklioğlu and Özen, 2007). Such an increase in salinity levels in freshwater ecosystems affects the dynamics and abundance of cladocerans especially keystone species *Daphnia* because they are very sensitive to changes in saline conditions (Akopian *et al*., 2002; Dodson and Frey, 2001; Wallace and Snell, 2001).

Since *Daphnia* has an important role in the freshwater food web, it is important to understand how it will be influenced by the stressors in the environment.

In this study, the aim was to determine the impacts of different global warming-triggerred stressor combinations on the survival and life history traits of *Daphnia pulex*. *Daphnia* individuals were collected from lake Eymir and cultured in the climate room. NaCl used as a salinity source and fish-exuded kairomone effect was obtained by incubation of *Alburnus alburnus,* which were also caught from Lake Eymir.

4.1 Acute Toxicity Experiments

In freshwater ecosystems, changes in the environmental conditions may affect the diversity of species. Salinity is one of the most important environmental variable for the freshwater organisms and in relation to this osmoregulation mechanism has significant impacts on the metabolism of freswater organisms (Lignot *et al*., 2000; Jeppesen *et al*., 2007). Acute toxicity experiments are used to determine the toxicant levels for the long-term chronic toxicity experiments. The amount of toxicant that members of Daphnidea withstand varies depending on the size of the speices. *Daphnia magna,* the largest daphnids, can resist high salinity levels (Lagersptez, 1955; Ranta, 1979; Bengtsson, 1986) as it was one of the most resistant among cladocers to the salinity increase. For *Daphnia magna*, 24 and 48 hours LC_{50} values of NaCl salinity were determined as 6.5 g/L and 4.5-5.5 g/L, respectively (Cowgill, 1987; Mount *et al*., 1997; EPA, 2007).

However, for the smaller caldocerans, LC_{50} vaules for NaCl were much lower, for example Cowgill and Milazzo (1991) and also Mount *et al*. (1997) found 24 and 48 hours LC_{50} for *Ceriodaphnia dubia were* 3.4 g/L and 2.0-2.5 g/L, respectively.

In the present study, daphnids were exposed not only to different salinity levels but also presence and absence of fish-exuded kairomone. Therefore at the beginning of this study, several acute toxicity tests were performed to obtain LC_{50} values for *Daphnia pulex* along with predation pressure effect. In all of acute toxicity experiment which had the highest doses,the aim was to determine the maximum resistance of daphnids to combine effects of high salinity levels and fish-exuded kairomone. For this purpose, we used extremely high salt levels in the first acute experiment (last two high doeses: 8.0 and 10.0 g/L). Whereas in the second and third acute toxicity experiments, the lower dose of salts were used which based on the experience gained in previous experiments.

In the absence of fish-exuded kairomone (F-), increased salinity decreased the survival of daphnids significanlty in all of the acute experiments (Table 3.1, 3.3, 3.5). Over the 2.5 g/L salinity level, nearly all of the daphnids were dead within 48 hours (Table 3.2, 3.4, 3.6, Figure 3.1, 3.2, 3.3). On the other hand, in the presence of fish-exuded kairomone (F+), except from 2 g/L salinity dose, survival of the animals were also decreased significantly. There weren't any significant difference obtained between the control group and 2 g/l salinity group. This result may imply that below 2 g/L of salinity, presence of fish-exuded kairomone enhance the resistance of daphnids to salnity. Thus, presence of fish-exuded kairomone, may have positive impacts on the survival under low-acute exposure of salinity. Whereas at higher salt levels, positive effect of fish predation dissapeared

Daphnia pulex collected from lake Eymir could not survive over 4 g/L of salt. This result supports the observation made by Sarma *et al*. (2006) that *D. pulex* which were collected from Mexican freshwater could not survive 4 g/L of salinity only for threre days. Besides it was revealed that Mexican freshwater cladocerans couldnot survive and reproduce in salinities over 5 g/L (Sarma *et al*., 2006). As mentioned before *D. pulex*, one of the small caldocerans, are more sensitive to the salinity than *D. magna* and it was thought that, while coping with salinity stress, *D. pulex* have similarities with the tropical cladoreans (Hall and Burns, 2002; Mohammed and Agard, 2007). Lilius (1995) found in an acute toxicity test with salinity that the 24 hours LC_{50} value was 3.0 g/L for *Daphnia pulex*. However, netither of the those studies included the impacts of fish-exuded kairomone along with the salt effect. Even though this value is higher than our highest salinity level, survival of the animals were declined significantly in our highest salinity level (Table 3.10, Figure 3.5-A&B, 3.6-A&B), furthermore, in our study presence of fish-exuded kairomone increased both 24 and 48 hours LC_{50} values. Therefore, it is hypothesed that when daphnids expose to fish-exuded kairomone, their resistence to salinity would increase. If the second stressor reduced the negative impact of the first one, this interaction is refferred ‘’antagonism’’ and this impact is also called ‘’reduced stress’’ (Folt *et al*., 1999). This is not widely recorded in the literature but Mason (2002) found that elements like calcium and copper had made the same effect and reduced the negative effects of lead, zinc and aliminum.

On the other hand in Lake Eymir, *D.pulex* individuals have to live together with fish predation pressure and unstable salinity conditions. Those conditions may have favored daphnids to be more resistant under the presence of fish-exuded kairomone.

4.2 Chronic Toxicity Experiments

Aim of the chronic toxicity test is to observe the responses of *Daphnia pulex* with long time exposure to the toxicant (Mason, 2002). Cladocerans are widely used in chronic toxicology studies to determine both population and individual level responses (Hall and Burns, 2002; Gama-Flores *et al*., 2004; Sarma and Nandini, 2006). Lethal Concentration$_{50}$ values, which are determined using acute toxicity experiments, are valuable to determine the sublethal impacts and to use in determining the chronic toxicity experiment doses. (Mason, 2002; Gama-Flores *et al*., 2004).

4.2.1 Survival Experiments

In this study, several acute toxicity experiments were performed to detect LC_{50} values for salt in the presense/absence of fish-exuded kairomone. As a result, in the presence of fish-exuded kairomone, 24-h LC_{50} value was found between 0.4-2.0 gl/L of salt. According to the OECD guidelines, the highest dose used for the chronic toxicity experiments should be close to the observed 24-h LC_{50} and the lowest dose should be 1/100 of the observed 24-h LC_{50} value (OECD, 1984). Consequently, salinity doses which were used in the chronic toxicity experiments were chosen between 0.00-1.5 g/L of salt.

In this part of the study, different combinations of stressors were employed to determine their impacts on the survival of *Daphnia pulex*, using chronic toxicity test procedures. Firstly, the combined effects of diffrent salinity doses and fish-exuded kairomone on the survival of *D. pulex* were investigated.

Freshwater organisms are hyperosmotic because of that thier inner-cell salinity is greater than the outer conditions (Lignot *et al*., 2000). To balance this situation, they exclude excess salt out of the cell, and take water inside by osmosis but salt can be toxic to freshwater life, interfering with basic ecological and physiological functions and adversely affecting species life histories and fitness, food supply, available habitat or breeding grounds (Jin, 2008). Large-sized *Daphnia*, which are the main controllers of phytoplankton in freshwater lakes (Carpenter and Kitchell, 1993), appear mainly at relatively low salinity (<2%) (Jeppesen *et al*., 1994). Survival of *D. pulex* individuals exposed to different NaCl doses in the absence (F-) and presence (F+) of fish-exuded kairomone significanlty reduced in the two higest doses (0.80 and 1.50 g/L) (Table 3.8, Figure 3.4-A&B). This is in line with findings of the others using *D. Magna.* .Increased salinity lead to increases in mortality (Teschner, 1995; Hall and Burns, 2002) and decreases in growth and reproduction (Baillieul, De Wachter and Blust, 1998).

In low doses (between 0.05-0.20 g/l salinity), the response of *D.pulex* was similar to the control group. These doses were probably too low for initiating a response different than the control treatment. Although at the intermnediate level of doses(0.40 g/l salinity) in the absence of fish-exuded kairomone survival of the individuals decreased significantly, suprisingly in the presence of fish-exuded kairomone the survival of the test organisms remined as high as the control group. Results of first chronic toxicity test supported the observations of acute toxicity tests that *D. pulex* individuals were more resistant to the salinity when the presence of fish-exuded kairomone. This finding is the first report in the literature to showed that combined effects of intermediate dose of salinity is insignificant provided that the test individuals are exposed to the salinity with fish-exuded kairomone on *Daphnia pulex.*

In the second survival experiment, the combined impacts of temperature, salinity, food availability and fish-exuded kairomone were examined.

In our region, Mediterranean Climatic zone, it is expected that, increase in the temperature through global warming may enhance the evaporation of water and this may increase the salinity levels in the freshwater systems (Zalidis *et al*., 2002; Beklioğlu and Tan, 2008) which is a limiting factor for *Daphnia* to survive as they lack an osmoregulatory system to minimize deleterious effect of excessive salt ions. In addition to its enhanced impact to salinisation, temperature increase also effects the algal community because warm summers with little vertical mixing provide ideal conditions for surface blooms of harmful cyanobacteria which are poor food for daphnids (Reynolds, 1997; Ibelings *et al*., 2003; Robson and Hamilton, 2003; Mooij *et al*., 2005) thus this will be a limiting factor to the diet of *Daphnia.* Fish predation pressure especially exerted from planktivorious fish is also expected to increase with global warming due to enhanced eutrophication (Beamish, 1995; Magnuson, Meisner and Hill, 1990; Shuter and Post, 1990; De Stasio *et al*., 1996), by increased anoxic conditions, affecting reproduction, changing the timing of resource peaks of piscivorous fish (Gillet, 1991; Gillet and Quetin, 2006; Straile, 2002).

It was observed that under the regular temperature treatment, survival of the animals decreased in the highest salinity level (1.5 g/L) within the low food levels but independent of fish-exuded kairomone (Table 3.10 Figure 3.5-A). However, under the high temperature treatment, survival of the animals sharply decreased independent of both salinity and fish predation pressure, (Table 3.10 Figure 3.5-B). It has been found that the survival and reproductivity of *D. pulex* and *D. pulicaria* individuals decreased significantly with the increased temperatures (Folt *et al*., 1999).

Temperature rises bring forward reproduction time, increase intrinsic growth rate, and shortens lifespan, all of this being a consequence of metabolic rate augmentation (Miracle and Serra, 1989; Serra *et al*., 1994). Furthermore, increased temperatures reduced the resistance of zooplankton to the salinity and enhanced mortality rates (Hall and Burns, 2002). Thus, our findings are in line with the literature.

Temperature may also influence fish–*Daphnia* interactions indirectly, by affecting the chemical signaling that plays a crucial role in this predator–prey system (Lass and Spaak, 2003). Our results are in accordance with the recent studies that the effects of high temperature, low food level and presence of fish exuded kairomone on the life history traits of *Daphnia* were synergistic (Weetman and Atkinson, 2002). But as the temperature increased, the effects of other stressors were masked, than it became the most important factor which influenced the survival of *Daphnia* as found in the study of Folt *et al*. (1999).

According to the results, presence of fish-exuded kairomone decreased survival of the animals, under the regular temperature treatment, in all salinity doses, with optimum and low food levels (Table 3.10, Figure 3.5-A, 3.6-B). Anti-predator defenses are often inducible and only employed in the presence of a predator (Tollrian and Harvell, 1999; Lass and Spaak, 2003) and they are effective in reducing mortality of zooplankton (Havel and Dodson, 1984). It has also been known that, kairomone reduces tolerance of *Daphnia* to environmental stress such as starvation (Hanazato, 1991a), high water temperature (Hanazato, 1991b), and pesticide combination (Hanazato and Dodson, 1992), and kairomone also reduce mortality (Havel and Dodson, 1984). However, in the previous acute and chronic survival experiments, it was found that presence of fish-exuded kairomone significantly increased the resistance of daphnids to saline conditions.

But it must be taken account that in those previous studies, only two different stressors (salinity and fish-exuded kairomone) were applied to the *Daphnia* individuals. So it could be expected that while trying to cope with more stressors, the response to the same stressors given in a large combination as a part of cocktail stresses can be different then mortality of the individuals could be enhanced by a response in a cocktail of effects as opposed to neutral or positive response given alone..

However, this experimental designed was not able show the negative effect of food levels. If food level itself is a stress factor, daphnids directly respond to but given with other stress factors such as temperature or salinity, it produced an antagonistic effect.

4.2.2 Life History Traits Experiment

In this study, the highest dose of acute toxicity experiment LD_{50} result was chosen as 1.5 g/L (in the absence/presence of fish-exuded kairomone: 24-h LD_{50} :0.401 g/L, 48-hLD_{50} : 0.159 g/L; and 24-h LD_{50} :1.962 g/L, 48-h LD_{50} : 1.007 g/L, respectively). But this highest dose (1.5 g/L) was the half of what had been observed by Lilius (1995) in an acute toxicity experiment. The reason could be that the resistance of saline conditions could differ between populations and even in clones of the animals (Hebert, 1987; Weider and Hebert, 1987; Mort, 1991;Ortells *et al*., 2005). Our finding were supported the previous survival experiment that , chronic exposure to the increased salinity, decreased the survival of daphnids significantly (Table 3.7, 3.8, 3.10). Except from the lowest salinity dose (0.2 g/L), most of the individuals were dead after eight day exposure of remaining salinity doses (Figure 3.7-A&B). Thus our results also supported that increasing salinities are mortal for the daphnids (Hall and Burns, 2002; Blinn *et al*., 2004).

In this study, daphnids were exposed to salinity and fish-exuded kairomone as in other acute and chronic survival toxicity experiments . But according to the results of this study, the effect of fish-exuded kairomone were not significant on the survival of daphnids but when it was considered with salinity, it reduced the survival significantly (Table 3.11).

In aquatic systems, zooplankton can mitigate the impacts of predators by behavioral, morphological and life-history responses that are triggered by a chemical cue (kairomone) exuded by the predator (De Meester *et al*., 1999; Tollrian and Dodson, 1999). These responses either reduce predation or allow high population growth to compensate for high predation (Zaret and Suffern, 1976; Stich and Lampert, 1981; Lampert, 1993).

Reproduction in crustaceans is strongly influenced by environmental factors of which the importance differs among species and among ecosystems (Sastry, 1983; Bouchon *et al.,* 1992) but it can be possible that, osmotic stress raised energy consumption for *Daphnia* and this may cause loss of energy for reproduction. Because fish feed visually and choose bigger individuals (Brooks and Dodson, 1965), *Daphnia* are tend to reproduce earlier and smaller to increase their chance to survive in the prescence of fish (Machácek 1991; Weber and Declerck 1997; Spaak *et al*. 2000; Sakwinska, 2002)., Reede (1995) also found that in the prescence of fish-exuded kairomone, age and size of first reproduction decreased and the number of eggs increased significantly. In accordance to that information, in this study, it was observed that, in the prescence of fish-exuded kairomone, daphnids tend to reproduce in smaller size and earlier age except the higest salinity dose (Table 3.14 and 3.15, Figure 3.9 and 3.10). Probably, the presence of fish-exuded kairomone affected the size and age at first reproduction, it was expected that presence of kairomone could affect the egg number which produced by daphnids (Sakwinska, 2002).

Salinity also had significant effect on the egg number of the daphnids (Table 3.12) thus there was a significant decrease in the egg number in the absence of fish-exuded kairomone within three high salinity doses (Table 3.13). In the presence of fish-exuded kairomone, egg number of the daphnids had the tendency of increase but this increase is not significant (Table 3.13, Figure 3.8). The reason could be that because especially higher salinity doses had significant negative impacts on the survival, daphnids couldn't had enough energy to cope with the stress of both the salinity and fish-exuded kairomone.

Under optimum conditions, *Daphnia* individuals use %27 of their energy for assimilation, %68 for reproduction and %5 for growth (Richman, 1958). Changes in the environmental conditions (predation pressure, increased salinity, temperature alteration, lower food availability, etc.) can change energy budget of the daphnids. When daphnids reach optimal body length, they use ¾ of their energy for reproduction (Richman, 1958; Baillieul *et al*., 1996). But with the increasing salinity daphnia individuals has to spend more energy to cope with stress and with the active transport mechanism, *Daphnia* individuals has to transport salt from outer to inner cell (Lignot, 2000). In the study, it was observed that body length of the individuals were significantly influenced with the interactions of time with salinity and fish-exuded kairomone while body width of the individuals were influenced with both salinity, fish-exuded kairomone and time (Table 3.16). When we considered the body length/body width ratio, it was also significant impacts of salinity and fish-exuded kairomone were observed (Table 3.17). Changes in the body shapes and lenghts were expected results as in the presence of fish-exuded kairomone but the changes observed in the absence of fish-exuded kairomone in higher salinity doses (Table 3.18) could be the response for the loss of water with using osmoregulation mechanism.

It could be suggested that although presence of fish-exuded kairomone affect the life history traits of *Daphnia*, salinity had also impacts on life history traits in additon to it's negative impacts on survival of the daphnids.

CHAPTER 5

CONCLUSION

In acute exposure to salinity, survival of the daphnids decreased significanlty over 2,5 g/L of salt. Presence of fish-exuded kairomone increased the resistance of daphnids to the intermadiate salinity doses thus this relationship seemed to be antagonistic. Results of first chronic toxicity test also supported the observations of acute toxicity tests that *D. pulex* individuals were more resistant to the salinity when exposed to fish-exuded kairomone. However over in high salinity doses, survival decreased both in the absence and presence of fish-exuded kairomone. In addition to the salinity, increase in the temperature and food limitation had negative impacts on the survival of the daphnids. Independent from other stressors (salinity, food limitation and presence of fish-exuded kairomone), temperature also reduced the survival significantly. Thus, the effect of temperature was enhanced with inclusion of food effect when given at optimum level, further reduced the survival.

As a result of the life history experiment, salinity had significant impact on the egg number thus in the presence of fish-exuded kairomone egg number were tend to be increased. Presence of fish-exuded kairomone decreased size at first reproduction significanlty. Age at first reproduction also decreased with the presence of fish-exuded kairomone but this decrease was not significant. Body length/ body with ratio also were significanlty affected by salinity and the presence of fish-exuded kairomone. The present study showed that the survival of the daphnids are significanlty affected by combination of multistressors and presence of fish-exuded kairomone and salinity affected the life history traits too.

In conclusion, *Daphnia* were very sensitive to the changes in the environmental conditions. As well as presence of multistressors which affected the survival and life history traits of daphnids, the impacts of those stressors may enhanced with the global warming period and this may have dreadful results on the food chain of the lake as it reduces the diversity of *Daphnia,* where it plays a major role as a an important grazer on the phytoplankton and a food source for fish.

CHAPTER 6

RECOMMENDATIONS FOR FUTURE STUDY

This work is particularly a preliminary study and right lead to future studies. Using a single clone may enhance the significance of the results. Furthermore, having a flow-through experimental setup may improve both cultural and experimantal conditions. For the future work, in accordance to the toxicology experiments, it may be more explanatory approach to investigate the effects of the stressors in molecular level.

REFERENCES

Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S., Rezaie, A., 2004. Pesticides and oxidative stress: a review. Med. Sci. Monit., Vol. 10, no:6, pp.141-147.

Adamo, S. A., Robert, D., Perez, J., Hoy R. R. 1995. The response of an insect parasitoid, Ormia ochracea (Tachinidae), to the uncertainty of larval success during infestation. Behav. Ecol. Sociobiol., Vol. 36, pp. 111–118.

Ahas, R., 1999. Long-term phyto-, ornitho- and ichthyophenological time-series analyses in Estonia. *International Journal of Biometeorology,* Vol. 42, no:3, pp. 119–123.

Akopian, M., Garnier, J., Pourriot, R., 2002. Cine´tique du zooplancton dans un continnum aquatique: de Marne et son re´ servoir a` l'estuaire de la Seine. C R Biol., Vol. 325,pp. 807–818.

Aladin, N.V., 1991. Salinity tolerance and morphology of the osmoregulation organs in Cladocera with special reference to Cladocera from the Aral sea. Hydrobiologia, Vol. 225, pp. 291–299.

Andersen, T., Elser, J. J., Hessen, D. O., 2004. Stoichiometry and population dynamics. Ecol. Lett., Vol. 7, pp. 884_ 900.

Andrewartha, H. G., Birch, L. C., 1984. The ecological web: more on the distribution and abundance of animals. – Univ. Chicago Press.

Arner, M., Koivisto S., 1993. Effects of salinity on metabolism and life history characteristics of *Daphnia magna.* Hydrobiologia, Vol. 259, pp. 69–77.

Arscott, D. B., Bowden, W. B., Finlay, J. C., 2000. Effects of desiccation and temperature/irradiance on the metabolism of 2 Arctic stream bryophyte taxa. Journal of the North American Benthological Society, Vol:19, no:2, pp. 263-273.

Baillieul, F., Fritig, B., Kauffmann, S.,1996. Occurrence among Phytophthora species of a glycoprotein eliciting a hypersensitive response in tobacco and its relationships with elicitins.. Mol. Plant- Microbe Interact., Vol. 9, pp. 214-216.

Baillieul, M., Selens M., Blust R., 1996. Scope for Growth and Fitness of *Daphnia magna* in Salinity-Stressed Conditions . *Functional Ecology*, Vol. 10, No. 2. pp. 227-233.

Baillieul, M., De Wachter, B., Blust, R., 1998. Effect of salinity on the swimming velocity of the water flea Daphnia magna. Physiological Zoology, Vol. 71, pp. 703-707.

Beamish, R. J., ed., 1995. *Climate Change and Northern Fish Populations.* Can. Spec. Publ. Fish. Aquat. Sci., Vol. *121*, pp. 737.

Becker, M., 1992. Ingestions- und Selektionsverhalten adulter Felchen (Coregonus Lavaretus) des. Bodensees: Saisonale und diurnale Variabilit€at, Dissertation Thesis. Universitat Konstanz, Konstanz, Germany.

Beisner, B. E., McCauley, E., Wrona, F. J., 1996. Temperature-mediated dynamics of planktonic food chains: the effect of an invertebrate carnivore. Freshwater Biology, Vol. 35, pp. 219-232.

Beisner, B. E., McCauley, E., Wrona, F. J., 1997. The influence of temperature and food chain length on plankton predator/prey dynamics. Can. J. Fish. Aquat. Sci., Vol. 54, pp. 586-595.

Beklioglu, M., Özen, A., 2007. Ulkemiz sıg gollerinde kuraklık etkisi ve ekolojik tepkiler. Uluslararası Kuresel Iklım Degisikligi ve Cevresel Etkiler Konferansı Bildiriler kitabı., pp. 299-306.

Beklioglu, M., Tan, C. O., 2008. Restoration of a shallow Mediterranean lake by biomanipulation complicated by drought. Fundamental and Applied Limnology / Archiv für Hydrobiologie, Vol. 171, no:2, pp. 105-118.

Bengtsson, L., Enell, M., 1986. Chemical analysis. In: Berglund BE (ed) Handbook of holocene paleoecology and palaeohydrology. Wiley, NY, pp 423–451.

Black, A. R., Dodson, S. I., 1990. Demographic costs of *Chaoborus*induced phenotypic plasticity in *Daphnia pulex*. *Oecologia*, Vol. 83, pp. 117–122.

Blinn, D. W., Halse, S. A., Pinder, A. M., Shie,l R. R,, McRae, J. M., 2004. Diatom and micro-invertebrate communities and environmental determinations in the western Australian wheatbelt: a response to salinization. Hydrobiologia, Vol. 528, pp. 229–248.

Boeing, W. J., 2002. Costs and benefits of *Daphnia* antipredator behavior and consequences on community stability. Dissertation,Louisiana State University.

Boersma, M., Spaak, P., De Meester, L. 1998. Predatormediated plasticity in morphology, life history, and behavior of *Daphnia*: the uncoupling of responses. Am. Nat., Vol. 152, pp. 237–248.

Bottrell, H. H., 1975. Generation time, length of instar, instar duration and frequency of moulting, and their relationship to temperature in eight species of Cladocera from the River Thames, Reading. *Oecologia*, Vol. 19, pp. 129–140.

Bouchon, D. C., Souty-rosset, J. Mocquard, P., Chentoufi, A., Juchault, P., 1992. Photoperiodism and seasonal breed- ing in aquatic and terrestrial Eumalacostraca.- Inver- tebrate Reproduction and Development, Vol. 22, pp. 203-212.

Breitburg, D. L., Sanders, J. G., Gilmour, C. C., Hatfield, C. A., Osman, R. W., Riedel, G. F., Seitzinger, S. P., Sellner, K. G., 1999. Variability in responses to nutrients and trace elements, and transmission of stressor effects through an estuarine food web. Limnol. Oceanogr., Vol. 44, pp. 837–863.

Brett, M. T., Müller-Navarra, D., Park, S. K., 2000. Empirical analysis of mineral P limitation's impact on algal food quality for freshwater zooplankton. Limnology and Oceanography, Vol. 45, pp. 1564-1575.

Brooks, J. L., Dodson, S., I., 1965. Predation, Body Size, and Composition of Plankton Science 1 October: Vol. 150, no:3692, pp. 28 – 35.

Brönmark, C., Miner, J. G., 1992. Predator-induced phenotypical change in body morphology in crucian carp. Science, Vol. 258, pp. 1348–1350.

Burns, C. W., 2000. Crowding-induced changes in growth, reproduction and morphology of *Daphnia*. Freshwater Biology, Vol. 43, pp. 19-29.

Calow, P., Sibly, R. M., 1990. A physiological basis of population processes: ecotoxicological implications. *Functional Ecology* , Vol. 4, pp. 283-288

Calow, P., 1991. Physiological costs of combating chemical toxicants: Ecological implications. Compo Biochem. Physiol., Vol. 100C, pp. 3-6.

Carpenter, S. R., Kraft, C. E., Wright, R., He, X., Soranno, P. A., Hodgson, J. R., 1992. Resilience and resistance of a lake phosphorus cycle before and after food web manipulation. American Naturalist, Vol. 140,no:781-798.

Carpenter, S. R., Kitchell, J. E., (eds) 1993. The trophic cascade in lakes. Cambridge Univ. Press.

Casey, R., Scrimgeour, G., Kendall, S., 2000. Final report: Effects of water temperature and treated pulp mill effluent on survival and growth of *Daphnia magna* (Cladocera: Daphnidae) and *Taenionema* (Plecoptera Taeniopterygidae)– Alberta Environment Sustainable Forest Management Research Program, Pubno: T/678.

Charmantier, G., Charmantier-Daures, M., 2001. Ontogeny of osmoregulation in crustaceans: the embryonic phase. *Am. Zool., Vol.* 41, pp. 1078-1089.

Cousyn, C., De Meester, L., Colbourne, J., Brendonck,K., Verschuren, L. D., Volckaert, F., 2001. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. Proceedings of the National Academy of Sciences, USA, Vol. 98, pp. 6256–6260.

Cowgill, J. M., 1987. Critical analysis of factors affecting the sensitivity of zooplankton and the reproducibility of toxicity test results, Water Res., Vol. 21, pp. 1453–1462.

Cowgill, U. M., Milazzo, D. P., 1991. The Sensitivity of Two Cladocerans to Water Quality Variables: Alkalinity. Archives of Environmental Contamination and Toxicology, Vol. 21, no:2 , pp. 224-232.

Das, S., Bose, A., Ghosh, B., 1995. Effect of salt stress on polyamine metabolism in *Brassica campestris*. Phytochemistry, Vol. 39, pp. 283–5.

Dawidowicz, P., Loose, C. J., 1992. Cost of swimming by *Daphnia* during diel verticalmigration. Limnol. Oceanogr., Vol. 37, pp. 665-669.

De Bernardi, R., Giussani, G., 1990. Are blue-green algae a suitable food for zooplankton? An overview. Hydrobiologia, Vol. 200/201, pp. 29–41.

De Coen, W. M., Janssen, C., 2003. The missing biomarker link: relationships
between effects on the cellular energy allocation biomarker of toxicantstressed
Daphnia magna and corresponding population characteristics.Environ. Toxicol. Chem., Vol. 22, pp. 1632–1641.

De Meester, L., Weider, L., 1999. Depth-selection behavior, fish kairomones, and the life histories of *Daphnia hyalina x galeata* hybrid clones. Limnology and Oceanography, Vol. 44, pp. 1248-1258.

DeMott, W. R., 1983. Seasonal succession in natural *Daphnia* assemblage. *Ecol. Monogr., Vol.* 53, pp. 321-340.

De Stasio, B. T., Jr, Hill D. K., Kleinhans J. M., Nibbelink N. P., Magnuson J. J., 1996. Potential effects of global climate change on small north temperate lakes: Physics, fishes and plankton. Limnology and Oceanography, Vol. 41, pp. 1136–1149.

Dodson, S. I., 1974. Adaptive change in plankton morphology in response to size selective predation: A new hypothesis of cyclomorphosis. Limnol. Oceanogr., Vol. 19, pp. 721-729.

Dodson, S. I.,1989. The ecological role of chemical stimuli for the zooplankton: Predator-induced morphology in *Daphnia*. Oecologia, Vol. 78, pp. 36 l-367.

Dodson, S. I., Wagner, A. M., 1996. Temperature affects selectivity of Chaoborus eating *Daphnia*. Hydrobiologia, Vol. 325, pp. 157-161.

Dodson, S. I., Frey, D. G., 2001. Cladocera and other branchiopoda. In: Thorp JH, Covich AP (eds) Ecology and classification of North American Freshwater invertebrates. Academic Press, London, pp 850–914.

Doksaeter, A., Vijverberg, J., 2001. The effects of food and temperature regimes on life-history responses to fish kairomones in *Daphnia hyalina* x *galeata*. Hydrobiologia, Vol. 442, pp. 207-214.

Downing, J. A., Rigler, F. H. (eds.), 1984. A Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters. Blackwell Scientific Publications, Oxford, England.

Dumont, H. J., Negrea, S. V., 2002. Introduction to the class Branchiopoda. Guides to the identification of the microcrustaceans of the continental waters of the world 19. Backhuys Publishers, Leiden, pp. 398.

Edmondson, W. T., Litt, A. H., 1982. *Daphnia* in Lake Washington. Limnol. Oceanogr., Vol. 27, pp. 272–293.

Elliott, J. E., Machmer, M., Wilson, L. K., Henny, C. J., 2000. Contaminants in Ospreys from the Pacific Northwest: II. Organochlorine pesticides, polychlorinated biphenyls and mercury, 1991-1997. Archives of Environmental Contaminants and Toxicology, Vol. 38, pp. 93-106.

Emmerson, M., Bezemer, M., Hunter, M. D., Jones, T. H., 2005. Global change alters the stability of food webs. Glob. Change Biol., Vol, 11, pp. 490–501.

EPA, 2007, last viewed 20 july 2008, http://www.epa.gov/pesticides/about/index.htm

Ferrao-Filho, A. S., Azevedo, S., 2003. Effects of unicellular and colonial forms of toxic *Microcystis aeruginosa* from laboratory cultures and natural populations on tropical cladocerans. Aquatic Ecology, Vol. 37, pp. 23-35.

Folt, C. L., Chen, C. Y., Moore, M., V., 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr., Vol. 44, pp. 864-877.

Frey, D. G., 1993. The penetration of cladocerans into saline waters. Hydrobiologia, Vol. 267, pp. 233–248.

Gama-Flores, J., L., Sarma, S. S. S. Nandini,' S., 2004. Acute and chronic toxicity of the pesticide methyl parathion to the rotifer *Brachionus angularis* (Rotifera) at different algal (*Chlorella vulgaris*) food densities ,Aquatic Ecology Volume 38, Number 1, pp. 27-36.

Garcia, C. M., Garcia-Ruiz, R., Rendon, M., Niell, F. X., Lucerna, J., 1997. Hydrological cycle and interannual variability of the aquatic community in a temporary saline lake (Fuente de Piedra, southern Spain). Hydrobiologia, Vol 345, pp. 131–141.

Genoni, G.P., 1997. Influence of the energy relationships of organic compounds on toxicity to the Cladocera Daphnia magna and the fish Pimephales promelas. Ecotoxicol. Environ. Saf., Vol. 36, no:1, pp. 27–37.

Gerten, D., Adrian, R., 2002. Species-specific changes in the phenology and peak abundance of freshwater copepods in response to warm summers. Freshwater Biology, Vol. 47, pp. 2163–2173.

Gießler, S., Mader, E., Schwenk, K., 1999. Morphological evolution and genetic differentiation in *Daphnia* species complexes. J. Evol. Biol., Vol. 12, pp. 710–723.

Gillet, C., 1991. Egg production in an Arctic charr (*Salvelinus alpinus* L.) brood stock: effects of temperature on the timing of spawning and the quality of eggs. Aquat. Living Resour., Vol. 4, pp. 109-116.

Gillet, C. and Quetin, P., 2006. Effect of temperature changes on the reproductive cycle of roach in Lake Geneva from 1983 to 2001. *J. Fish. Biol.*, Vol. 69, pp. 518—534.

Gliwicz, Z. M., 2003. Between Hazards of Starvation and Risk of Predation: The Ecology of Offshore Animals. Excellence in ecology, Book 12, International Ecology Institute, Oldendorf/Luhe.

Goldoni, M., Johansson, C., 2007. A mathematical approach to study combined effects of toxicants in vitro: Evaluation of the Bliss independence criterion and the Loewe additivity model. Toxicol. In Vitro, Vol. 21, pp. 759–769

Goulden, C. E., Comotto, R. M., Henderickson, J. A., Henry, JR. L. L., Johnson, K. L., 1982. Procedures and recommendations for the culture and use of *Daphnia* in bioassay studies.. In Aquatic toxicology and hazard assessment. 5th Conf. Am. Sot. Testing Mater. ASTM STP, Vol. 766, pp. 148- 169.

Goss, L. B., Bunting D. L., 1983. *Daphnia* development and reproduction responses to temperature. *Journal of Thermal Biology*, Vol. 8, pp. 375–380.

Green, J., 1993. Zooplankton associations in East African lakes spanning a wide salinity range. Hydrobiologia, Vol. 267, pp. 249–256.

Green, J., Menengestou, S., 1993. Specific diversity and community structure of Rotifera in a salinity series of Ethiopian inland waters. Hydrobiologia, Vol. 209, pp. 95–106.

Grzesiuk, M., Mikulski, A., 2006. The effect of salinity on freshwater crustaceans Polish Journal of Ecology, Vol: 54, no: 4, pp. 669-674.

Gulati,, R. D., Demott, W. R.,1997. The role of food quality for zooplankton: Remarks on the state-of-the-art, perspectives and priorities. Freshwater Biol., Vol. 38, pp. 753–768.

Hall, C., Burns, C. W., 2002. Mortality and growth responses of *Daphnia carinata* to increases in temperature and salinity. Freshwater Biology, Vol. 47, pp. 451-458.

Hammer, U. T., 1993. Zooplankton distribution and abundance in saline lakes of Alberta and Saskatchewan, Canada. Int J Salt Lake Res., Vol. 2, pp. 111–132

Hanazato, T., 199lb. Effects of a *Chaoborus*-released chemical on *Daphnia ambigua*: Reduction in the tolerance of the *Daphnia* to summer water temperature. Limnol. Oceanogr., Vol. 36, pp. 165-171.

Hanazato, T., 1991 a. Influence of food density on the effects of a *Chaoborus*-released chemical on *Daphnia ambigua*. Freshwater Biol., Vol. 25, pp. 477-483.

Hanazato, T., Dodson, S. I., 1992. Complex effects of a kairomone of *Chaoborus* and an insecticide on *Daphnia pulex*, Journal of plankton research, Vol. 14, pp. 1743-1755.

Hanazato, T., Fueki, K., Yoshimoto, M., 2001. Fish-induced lifehistory shifts in the cladocerans Daphnia and Simochephalus: are they positive or negative responses? J. Plankton Res., Vol. 23, pp. 945–951.

Haney, J. F., 1993. Environmental control of diel vertical migration behaviour. *Arch. Hydrobiol. Beih. Ergebn. Limnol.*, Vol. 39, pp. 1–17.

Harrington, R., Woiwod, I., Sparks, T., 1999. Climate change and trophic interactions. Trends in Ecology and Evolution, Vol. 14, pp. 146-150.

Hart, R. C., McLaren, I. A. ,1978. Temperature acclimation and other influences on embryonic duration in the copepod *Pseudocalanus sp.* Journal of marine biology, Vol. 45, pp. 1.

Hart, B. T., Bailey, P., Edwards, R., Hortle, K., James, K., McMahon, A., Meredith, C., Swadling, K., 1991. A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia, Vol. 210, pp. 105–144.

Harvell, C. D., 1986. The ecology and evolution of inducible defenses in a marine bryozoan: Cues, costs, and consequences. Am. Nat., Vol. 128, pp. 810-823.

Havel, J. E., Dodson, S., I., 1984. *Chaoborus* predation on typical and spined morphs of *Daphnia pulex*: behavioral observation. Limnology and Oceanography, Vol. 29, pp. 487–494.

Havel, J. E., Dodson, S. I., 1987. Reproductive costs of *Chaoborus* induced polymorphism in *Daphnia pulex*. Hydrobiologia, Vol. 150, pp. 273–281.

Havel, J. E., 1985. Cyclomorphosis of *Daphnia pulex* spined morphs. *Limnology and Oceanography, Vol.* 30, pp. 853–861.

Hebert, P. D. N., 1987. Genotypic characteristics of the Cladocera. Hydrobiologia, Vol. 145, pp. 183-193.

Heugens, E., 2003. Predicting effects of multiple stressors on Aquatic Biota December 17 ISBN 90-76894-40-X pp. 167.

Hessen, D. O., 1992. Nutrient element limitation of zooplankton production. Am. Nat., Vol. 140, pp. 799–814.

Hrbajek, J., 1977. Competition and predation in relation to species composition of freshwater zooplankton, mainly Cladocera,. In J. Cairns [ed.], Aquatic microbial communities. Garland., pp. 305-353.

Hu, S. S., Tessier, A. J., 1995. Seasonal succession and the strength of intra and interspecific competition in a *Daphnia* assemblage. Ecology, Vol. 76, pp. 2278–2294.

Ibelings, B. W., Vonk, M., Los, H. F. J., Van Der Molen, D., Mooij, W. M., 2003. Fuzzy modeling of cyanobacterial surface waterblooms: validation with NOAA-AVHRR satellite images. *Ecol Appl.*, Vol. 13, pp. 1456-1472.

Innes, D. J., Singleton, D. R., 2000. Variation in allocation to sexual and asexual reproduction among clones of cyclically parthenogenetic *Daphnia pulex* (Crustacea: Cladocera). Biological Journal of the Linnean Society, Vol. 71, pp. 771–787.

IPCC Assessment Report 2001, viewed 28 july 2008, http://www.ipcc.ch/

IPCC Assessment Report 2007, viewed 28 july 2008, http://www.ipcc.ch/

Jeppesen, E., Søndergaard, M., Kanstrup, E., Petersen, B., Henriksen, R.B., Hammershøj, M., Mortensen, E., Jensen, J.P., Have, A. 1994. Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ? Hydrobiologia, Vol. 275-276, pp. 15-30.

Jeppesen, E., Jensen, P. J., Søndergaard, M., Lauridsen, T. L., 1999. Trophic dynamics in turbid and clearwater lakes with special emphasis on the role of zooplankton for water clarity. Hydrobiologia, Vol. 408/409, pp. 217–231.

Jeppesen, E., Søndergaard, M., Meerhoff, M., Lauridsen, T. L., Jensen, J. P., 2007. Shallow lake restoration by nutrient loading reduction - some recent findings and challenges ahead. *Hydrobiologia, Vol.* 584, pp. 239-252.

Jespersen, A. M., Christoffersen, K., 1987. Measurement of chlorophyll-a from phytoplankton using ethanol as extraction solvent. Arch. Hydrobiol., Vol. 109, pp. 445-454.

Jin, C., 2008. Biodiversity dynamics of freshwater wetland ecosystems affected by secondary salinisation and seasonal hydrology variation: a model-based study *Hydrobiologia., Vol.* 598, no:1, p.257.

Johnston, I. A., Bennett, A. F., 1996. *Animals and temperature: Phenotypic and evolutionary adaptation.* Cambridge University Press, Cambridge.

Kefford, B. J., P. J. Papas, L. Metzeling, Nugegoda D., 2004. Do laboratory salinity tolerances of freshwater animals correspond with their field salinity? Environmental Pollution, Vol. 129, pp. 355–362.

Kefford, J., Fields Elizabeth, J., Clay, C., Dayanthi, N., 2007. Salinity tolerance of riverine microinvertebrates from the southern Murray–Darling Basin. *Marine and Freshwater Research,* Vol. 58, no:11, pp. 1019–1031.

Kerfoot, W. C., Sih, A., 1987. Predation: Direct and Indirect Impacts on Aquatic Communities. University Press of New England, Hanover,Germany, and London.

Kilham, S. S., Kreeger, D. A., Lynn, S. G., Goulden, C. E., Herrera, L., 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia, Vol. 377, pp. 147-159.

Krist, A. C., 2001. Variation in fecundity among populations of snails is predicted by prevalence of castrating parasites. Evol. Ecol. Res., Vol. 3, pp. 191–197.

Krueger, D. A., Dodson, S. I., 1981. Embryological induction and predation ecology in *Daphnia pulex*. *Limnol. Oceanogr.*, Vol. 26, pp. 219–223.

Kuhlmann, H. W., Kusch, J., Heckmann, K., 1999. Predator-induced defences in ciliated protozoa. In Tollrian, R. And Harvell, C. D.(eds), The Ecology and Evolution of Inducible Defences. Princeton University Press, Princeton, pp. 142-159.

Lagerspetz, K., 1955. Physiological studies on the brackish water tolerance of some species of *Daphnia*. Arch. Soc. Vanamo. Suppl., Vol.9, pp. 138–143.

Lampert, W. 1977a,b. Studies on the carbon balance of *Daphnia pulex* as related to environmental conditions. 1. Methodological problems of the use of 14C for the measurement of carbon assimilation. 2. The dependence of carbon assimilation on animal size, temperature, food concentration and diet species. Arch. Hydrobiol. Suppl., Vol. 48, pp. 287-307, 310-335.

Lampert, W., (ed.) 1985. Food limitation and the structure of zooplankton communities. Arch. Hydrobiol. Beih. Ergebn. Limnol., Vol. 21, pp. 497.

Lampert, W., Fleckner, W., Rai, H., Taylor, B. E., 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. Limnol. Oceanorg., Vol. 31, pp. 478-490.

Lampert, W., 1993. Phenotypic plasticity of the size at first reproduction in *Daphnia*—the importance of maternal size. Ecology, Vol. 74, pp. 1455–1466.

Lampert, W., 2006. "*Daphnia*: Model herbivore, predator and prey." Polish Journal of Ecology, Vol. 54, no:4, pp. 607-620.

Larsson, P., Dodson, S., 1993. Invited review – chemical communication in planktonic animals. Arch Hydrobiol, Vol. 129, pp. 129–155.

Lass, S., Spaak, P., 2003. Chemically induced anti-predator defenses in plankton: a review. Hydrobiologia, Vol. 491, pp. 221–239.

Lignot, J. H., Spanings-Pierrot, C., Charmantier, G., 2000. Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. Aquaculture, Vol. 191, pp. 209-245.

Lilius, H., Hastbacka, T., Isoma B., 1995. A comparison of the toxicity of 30 reference chemicals to *Daphnia magna* and *Daphnia pulex*. Environ. Toxicol. Chem., Vol.14, pp. 2085-2088.

Loose, C. J. ,1993. *Daphnia* diel vertical migration behavior: Response to vertebrate predator abundance. Ergeb. Limnol., Vol. 39, pp. 29-36.

Loose, C. J., von Elert, E., Dawidowicz, P., 1993. Chemically-induced diel vertical migration in *Daphnia*: a new bioassay for kairomones exuded by fish. Arch. Hydrobiol., Vol. 126, pp. 329-337.

Loose, C. J., Dawidowicz, P.. 1994. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance – Ecology, Vol. 75, pp. 2255-2263

Lüning, J. 1992. Phenotypic plasticity of *Daphnia pulex* in the presence of invertebrate predators: morphological and life history responses. Oecologia, Vol.92, pp. 383–390.

Lynch, M., 1978. Complex interactions between natural coexploiters – *Daphnia* and *Ceriodaphnia*. Ecology, Vol. 59, pp. 552–564.

Lynch, M., 1980. Aphanixomenon blooms: Altcrnatc control and cultivation by *Daphnia pulex*. Am. Sot. Limnol. Oceanogr. Spec. Symp., Vol. 3, pp. 299-304.

Machácek, J., 1991. Indirect effect of planktivorous fish on the growth and reproduction of *Daphnia galeata*. Hydrobiologia, Vol. 225, pp. 193–198.

Macháček, J., 1993. Comparison of the response of *Daphnia galeata* and *Daphnia obtuse* to fish-produced chemical substance. Limnol. Oceanogr., Vol. 38, pp. 1544–1550.

Magnuson, J. J., Meisner, J. D., Hill, D.K., 1990. Potential changes in the thermal habitat of Great Lakes fish after global climate warming. Transactions of the American Fisheries Society. Vol. 119, pp. 254-264.

Martin-Creuzburg, D., Wacker, A., Von Elert, E., 2005. Life history consequences of sterol availability in the aquatic keystone species *Daphnia*. Oecologia, Vol, 144, pp. 362–372.

Martinez-Jeronimo, F., Martinez-Jeronimo, L., 2007. Chronic effect of NaCl salinity on a freshwater strain of *Daphnia magna Straus* (Crustacea: Cladocera): A demographic study. Ecotoxicology and Environmental Safety. Vol. 67, pp. 411–416.

Mason, C. F., 2002. Biology of freshwater pollution. 4th Ed. Harlow, England: Pearson Education Limited.

Mehner, T., 2000. Influence of spring warming on the predation rate of underyearling fish on Daphnia—a deterministic simulation approach. Freshwater Biol., Vol. 45, pp. 253–263.

Millennium Ecosystem Assessment, 2005. last viewed 20 july 2008. http://www.maweb.org/en/index.aspx

Mikulski, A., 2001. The presence of fish induced the quick release of offspring by *Daphni*a. Hydrobiologia, Vol. 442, pp. 195–198.

Miracle, M. R., Serra, M., 1989. Salinity and temperature influence in rotifer life history characteristics. Hydrobiologia, Vol. 186/187, pp. 81- 102.

Mohammed, A., Agard, J. B. R., 2007. Comparative Salinity Tolerance of Three Indigenous Tropical Freshwater Cladoceran Species; Moinodaphnia Macleayi, Ceriodaphnia Rigaudii and Diaphanosoma Brachyurum Environmental Monitoring and Assessment, Volume 127, , pp. 1-3.

Mooij, W. M., Hülsman, S., De Senerpont Domis, L. N,. 2005. The impact of climate change on lakes in the Netherlands: a review. Aquatic Ecology, Vol. 39, pp. 381–400.

Moore, M. V., Folt, C. L., 1993. Zooplankton body size and community structure: effects of hermal and toxicant stress. Trends Ecol. Evol., Vol. 8, pp. 178-183.

Moore, M. V., Folt, C. L., Stemberger, R. S., 1996. Consequences of elevated temperatures for zooplankton assemblages in temperate lakes. *Archiv für Hydrobiologie*, Vol. 135, pp. 289–319.

Mort, M. A., 1991. Bridging the gap between ecology and genetics: the case of freshwater zooplankton. Trends in Ecology and Evolution, Vol. 6, pp. 41-45.

Mount, D. R., Gulley, D. D., Hockett, J. R., Garrison, T. D., Evans, J. M., 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia, Daphnia magna* and *Pimephales promelas* (flathead minnows). Environmental Toxicology and Chemistry, Vol. 16, pp. 2009–2019.

Munro, I. G., 1974. The effect of temperature on the development of egg, naupliar and copepodite stages of two species of copepods, *Cyclops vicinus Uljianin* and *Eudiaptomus gracilis* Sars. Oecologia, Vol. 16, pp. 335-367.

Müller-Navarra, D. C., Brett, M. T., Liston, A., Goldman, C. R., 2000. A highly-unsaturated fatty acid predicts biomass transfer between primary producers and consumers. Nature. Vol. 403, pp. 74-77.

Nogrady, T., Wallace, R. L., Snell, T. W., (eds) 1993. *Rotifera—guides to the identification of the microinvertebrates of the continental waters of the world*, 4. SPB Academic Publishing, The Hague, pp. 142.

OECD., 1984. *Daphnia sp.*, acute immobilisation test and reproduction test. Part I - 24h EC50 acute immobilisation test. Organization of Economic Co-operation and Development, Paris, France.

OECD, 1998. OECD guideline for testing chemicals 211. *Daphnia magna* reproduction test. Organization of Economic Co-operation and Development, Paris, France..

OECD., 2004. *Daphnia sp.,* acute immobilization test. In: OECD guidelines for testing of chemicals, vol. 202. Paris: Organization for Economic Cooperation and Development.

Orcutt, J. D., Porter, K. G., 1983. Diel vertical migration by zooplankton: constant and fluctuating temperature effects on life history parameters of *Daphnia*. Limnology and Oceanography, Vol. 28, pp. 720-730.

Ortells, R., Reusch, T. B. H., Lampert, W., 2005. Salinity tolerance in *Daphnia magna*: characteristics of genotypes hatching from mixed sediments. Oecologia, Vol.143, pp. 509-516.

Petchey, O. L., McPhearson, P. T., Casey, T. M., Morin, P. J., 1999. Environmental warming alters food-web structure and ecosystem function. Nature, Vol. 402, pp. 69-72.

Pijanowska, J., 1997. Alarm signals in *Daphnia*. Oecologia, Vol. 112, pp. 12–16.

Polak, M., Starmer, W. T., 1998. Parasite-induced risk of mortality elevates reproductive effort in male *Drosophila* Proc. R. Soc. B., Vol. 265, pp. 2197–2201.

Peredo-Alvarez, V. M., Sarma, S. S. S., Nandini, S., 2003. Combined effect of concentrations of algal food (*Chlorella vulgaris*) and salt (sodium chloride) on the population growth of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera). Rev. Biol. Trop., Vol. 51, pp. 399–408.

Ranta**,** E., 1979. Population biology of *Darwinula stevensoni* (Crustacea, Ostracoda) in an oligotrophic lake. Annales Zoologici Fennici Vol. 16, pp. 28-35.

Ramdani, M., Elkhiati, N., Flower, R. J., 2001. Open water zooplankton communities in north African wetland lakes: the CASSARINA project. Aquat. Ecol., Vol. 35, pp. 319–333.

Reede, T., 1995. Life history shifts in response to different levels of fish kairomones in *Daphnia*. J. Plankton Res., Vol. 17, pp. 1661–1667.

Reichwaldt, E. S., 2008. Food quality influences habitat selection in *Daphnia*. Freshwater Biology, Vol. 53, pp. 872-883.

Riessen, H. P., Sprules, W. G., 1990. Demographic costs of antipredator defenses in *Daphnia pulex*. Ecology, Vol. 71, pp. 1536–1546.

Reynolds, C. S., 1984. The Ecology of Freshwater Phytoplankton, Cambridge University Press, Cambridge.

Reynolds, C. S., 1997. Vegetation Processes in the Pelagic: A Model for Ecosystem Theory. Excellence in Ecology (9th edn). Ecology Institute, Oldendorf/Luhe.

Ricciardi, A., Rasmussen, J. B., 1999. Extinction rates of North American freshwater fauna. Conservation Biology, Vol. 13, pp. 1220-1222.

Richman, S., 1958. The transformation of energy by *Daphnia pulex*. Ecol. Monogr., Vol. 28, pp. 273-291.

Riessen, H. P., 1999 . Predator-induced life history shifts in *Daphnia*: a synthesis of studies using meta-analysis. Can. J. Fish. Aquat. Sci., Vol. 56, pp. 2487–2494.

Ringelberg, J., 1991. Enhancement of the phototactic reaction in *Daphnia hyalina* by a chemical mediated by juvenile perch (*Perca fluviatilis*). J. Plankton Res., Vol. 13, pp. 17–25.

Ringelberg, J., Van Gool, E., 1998. Do bacteria, not fish, produce 'fish kairomone'? J. Plankton Res., Vol. 20, pp. 1847–1852.

Robson, B. J., Hamilton, D. P., 2003. Summer flow event induces a cyanobacterial bloom in a seasonal Western Australian estuary. Mar. Freshwater Res., Vol. 54, pp. 139–151.

Sakwinska, O., 1998. Plasticity of *Daphnia magna* life history traits in response to temperature and information about a predator. Freshw. Biol., Vol. 39, pp. 681–687.

Sakwinska, O., 2002. Response to fish kairomone in *Daphnia galeata* life history traits relies on shifts to earlier instar at maturation. Oecologia, Vol. 131, pp. 409–417.

Sala, O. E., Chapin III, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., 14 others, 2000. Global Biodiversity Scenarios for the Year 2100. *Science, Vol.* 287.

Sarma, S. S. S. , Elguea-Sanchez, B., Nandini, S., 2002. Effect of salinity on competition between the rotifers *Brachionus rotundiformis Tschugunoff* and *Hexarthra jenkinae jenkinae* (De Beauchamp) (Rotifera). Hydrobiologia, Vol.
474, pp. 183–188.

Sarma, S. S. S., Beladjal, L., Nandini. S., Ceron-Martinez, G., Tavera-Briseno, K., 2005. Effect of salinity stress on the life history variables of *Branchipus schaefferi* Fisher, 1834 (Crustacea: Anostraca). Saline Syst., Vol. 1,no:4, pp. 1–11.

Sarma, S. S. S., Nandini, S., Morales-Ventura, J., Delgado-Martinez, I., Gonzalez-Valverde, L, 2006. Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans Aquat. Ecol., Vol. 40, pp. 349–360.

SAS, Version 9.00

Sastry, A. N., 1983. Pelagic larval ecology and development. In: Vernberg, F.J. (Ed.), The Biology of Crustacean, vol. 8. Academic Press, New York, pp. 213– 282.

Schallenberg, M., Bremer, P. J., Henkel, S., Launhardt, A., Burns, C. W., 2005. Survival of *Campylobacter jejuni* in Water: Effect of Grazing by the Freshwater Crustacean *Daphnia carinata* (Cladocera) Appl. Environ Microbiol., Vol. 71, no:9 , pp. 5085–5088.

Scheffer, M.i 1999. The effect of aquatic vegetation on turbidity; how important are the filter feeders? Hydrobiologia, Vol. 408/409, pp. 307–316.

Schriver, P., Bøgstrand, J., Jeppesen, E., Søndergaard, M., 1995. Impact of submerged macrophytes on fishzooplankton– phytoplankton interactions: large-scale enclosure experiments in a shallow eutrophic lake. Freshwater Biology, Vol. 33, pp. 255–270.

Serra, M., Carmona, M. J., Miracle, M. R., 1994. Survival analysis of three clones of *Brachionus plicatilis* (Rotifera). *Hydrobiologia, **XT7***, pp. 97-105.

Shuter, B. J., Post, J. R., 1990. Climate, population viability and the zoogeography of temperate fishes. Transactions of the American Fisheries Society, Vol. 119, pp. 314-336.

Simcic, T., Brancelj, A., 1997. Electron transport system (ETS) activity and respiration rate in five *Daphnia* species at different temperatures. Hydrobiologia, Vol. 360, pp. 117–125.

Smolders, R., Baillieul, M., Blust, R., 2005. Relationship between the energy status of *Daphnia magna* and its sensitivity to environmental stress. *Aquat. Toxicol, Vol..* 73, pp. 155-170.

SPSS Statistical Software Version 13.0 for Windows, SPSS Inc.

Spaak, P., Vanoverbeke, J., Boersma, M., 2000. Predator induced life history changesand the coexistence of five taxa in a *Daphnia* species complex. Oikos, Vol. 89, pp. 164-174.

Spitze, K., 1991. *Chaoborus* predation and life-history evolution in *Daphnia pulex*: temporal pattern of population diversity, fitness, and mean life history. *Evolution*, Vol. 45, pp. 82–92.

Sterner, R. W., Schulz, K. L., 1998. Nutrition: Recent progress and a reality check. Aquat. Ecol., Vol. 32, pp. 261–279.

Stich, H. B., Lampert, W., 1981. Predator evasion as an explanation of diurnal vertical migration. *Nature, Vol.* 293, pp. 396–398.

Stich, H. B., Lampert, W., 1984. Growth and reproduction of migrating and nonmigrating *Daphnia* species under simulated food and temperature conditions of diurnal vertical migraiton. Oecologia, Vol. 61, pp. 192-196.

Strecker, U., Faúndez, V., Wilkens, H., 2004. Phylogeography of surface and cave *Astyanax* (Teleostei) in Upper Central America based on cytochrome *b* sequence data. Mol. Phylogenet. Evol., Vol. 33, pp. 469–481.

Stibor, H., 1992. Predator-induced life-history shifts in a freshwater cladoceran. Oecologia, Vol. 192, pp. 162–165.

Straile, D., 2000. Meteorological forcing of plankton dynamics in a large and deep continental European lake. Oecologia,Vol. 122, pp. 44-50.

Straile, D., 2002. North Atlantic Oscillation synchronizes food-web interactions in central European lakes. *Proc. R. Soc. Lond. B.*, Vol. 269, pp. 391-395.

Talling, J. F., 2003. Phytoplankton-zooplankton seasonal timing and clear-water phase in some English lakes. Freshwater Biol., Vol. 48, pp. 39–52.

Teschner, M., 1995. Effects of salinity on the life history and fitness of *Daphnia magna*: variability within and between populations. Hydrobiologia, Vol. 307, pp. 33-41.

Thomas, C., Cameron, D., Green, A., Bakkenes, R. E., Beaumont, M., Collingham, L. J., Erasmus, B. F. N., De Siqueira, Y. C., Grainger, M. F.,

Hannah, A., Hughes, L. L., Huntley, B., Van Jaarsveld, A. S., Midgley, G. F., Miles, L., Ortega- Huerta, M. A., Peterson, A. T., Phillips, O. L., Williams, S. E., 2004. Extinction risk from climate change. *Nature*, Vol. 427, pp. 145-148.

Thornhill, J. A., Jones, J. T., Kusel, J. R., 1986. Increased oviposition and growth in immature *Biomphalaria glabrata* after exposure to *Schistosoma mansoni*. Parasitology, Vol. 93, pp. 443–450.

Tilman, D., 1999. The ecological consequences of changes in biodiversity: a search for general principles. The Robert H. MacArthur Award Lecture. *Ecology, Vol. 80, pp. 1455-1474.*

Tollrian, R., 1993. Neckteeth formation in *Daphnia pulex* as an example of continuous phenotypic plasticity: Morphological effects of *Chaoborus* kairomone concentration and their quantification. J. Plankton Res., Vol. 15, pp. 1309-1318.

Tollrian, R., Dodson, S., I., 1999. Inducible defences in cladocera, constraints, costs, and multi-predator environments. in Tollrian & Harvell (eds) The ecology and evolution of inducible defenses. Princeton University Press, Princeton New Jersey.

Tollrian, R., Harvell, C. D., 1999. The ecology and evolution of inducible defenses. Princeton University Press, Princeton New Jersey.

Urabe, J., Clasen, J., Sterner, R. W., 1997. Phosphorus limitation of *Daphnia*: Is it real? Limnology and Oceanography, Vol. 42, pp. 1436-1443.

Van Buskirk, J., Schmidt, B. R., 2000. Predator-induced phenotypic plasticity in larval newts: trade-offs, selection, and variation in nature. Ecology, Vol. 81, pp. 3009–3028.

Vijverberg, J., 1989. Culture techniques for studies on the growth, development and reproduction of copepods and cladocerans under laboratory and in situ conditions: A review. Freshwater biology. Oxford [Freshwat. Biol.]., Vol. 21, no:3, pp. 317-373.

Vinebrooke, R. D., and others, 2003. Trophic dependence of ecosystem resistance and species compensation in experimentally acidified Lake 302S (Canada). Ecosystems, Vol. 6, pp. 101–113.

Visser, M. E., Both, C., 2005. Shifts in phenology due to global climate change: the need for a yardstick. Proc. R. Soc. Lond. Ser. B. Bio.l Sci DOI:10.1098/rspb.2005.3356.

Von Elert, E., Pohnert, G., 2000. Predator specificity of kairomones in diel vertical migration of *Daphnia*: a chemical approach. Oikos, Vol. 88, no:1, pp. 119-128.

Von Elert, E., 2002. Determination of limiting polyunsaturated fatty acids in Daphnia galeata using a new method to enrich food algae with single fatty acids. Limnol. Oceanogr., Vol. 47, pp. 1764–1773.

Wagner, A., Benndorf, J., 2007. Climate-driven warming during spring destabilises a *Daphnia* population: a mechanistic food-web approach. *Oecologia*, Vol. 151, pp. 351-364.

Wallace, R. L., Snell, T. W., 2001. Phylum Rotifera. In: Thorp JH, Covich AP (eds) Ecology and classification of North American freshwater invertebrates. Academic Press, 2nd edn. New York, pp 195–254.

Ward, J. V., Stanford, J. A., 1982. Thermal responses in the evolutionary ecology of aquatic insects. *Annual Review of Entomology*, Vol. 27, pp**.** 97–117.

Weber, A., Declerck, S., 1997. Phenotypic plasticity of *Daphnia* life history traits in response to predator kairomones: genetic variability and evolutionary potential. Hydrobiologia, Vol. 360, pp. 89-99,

Weetman, D., Atkinson, D., 2002. Antipredator reaction norms for life history traits in *Daphnia pulex*: dependence on temperature and food. Oikos, Vol. 98, pp. 299–307.

Weider, L. J., Hebert, P. D. N., 1987. Ecological and physiological ifferentiation among low-arctic clones of *Daphnia pulex*. *Ecology, Vol.* 68, pp. 188-198.

Weider, L. J., Pijanowska, J., 1993. Plasticity of *Daphnia* life histories in response to chemical cues from predators. Oikos, Vol. 67, pp. 385–392.

Wetzel, R. G., 1983. Limnology. 2nd edition. -Saunders College Publishing, New York, pp. 1-767.

Williams, W. D., 1987. Salinization of rivers and streams: an important environmental hazard. Ambio., Vol.16, pp. 180–185.

Williams, W. D., De Deccker, P., Shiel, R., J., 1998. The limnology of Lake Torrens, an episodic salt lake of Central Australia, with particular reference to unique events in 1989. Hydrobiologia, Vol. 384, pp. 101–110.

Wilson, A. E., Sarnelle, O., Tillmanns, A. R., 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments*. Limnology and Oceanography, Vol.* 51, no:4, pp. 1915-1924.

Winder, M., Schindler, D. E., 2004. Climatic effects on the phenology of lake processes. Global Change Biol. Vol., 10, pp. 1844–1856.

Young, G., Björnsson, B. T., Prunet, P., Lin, R. J., Bern, H. A., 1989. Smoltification and seawater adaptation in coho salmon (*Oncorhynchus kisutch*): plasma prolactin, thyroid hormones, and cortisol. *General and Comparative Endocrinology, Vol.* 74, pp. 335–345.

Zalidis, G., Stamatiadis, S., Takavakoglou, V., Eskridge, K., Misopolinos, N., 2002. Impacts of agriculrural practices on soil and water quality in the Mediterranean region and proposed assessment methodology. Agric. Ecosys. Environ., Vol. 88, pp. 137-146.

Zaret, T. M., Suffern, J. S., 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnol. Oceanogr., Vol. 21, pp. 804-813.

Zaret, T. M., 1980. Predation and freshwater communities. Yale Univ. Press, New Haven, Connecticut., pp. 187.