

The rotifer *Brachionus plicatilis*: an emerging bio-tool for numerous applications

Venetia KOSTOPOULOU^{1*}, María José CARMONA² and Pascal DIVANACH¹

¹ Institute of Aquaculture, Hellenic Centre for Marine Research,
P.O. Box 2214, Heraklion, 71003 Crete, Greece

² Institut Cavanilles de Biodiversitat i Biologia Evolutiva, Universitat de València,
P.O. Box 22085, 46071 Valencia, Spain

Received: 5 May 2011

Accepted after revision: 7 September 2011

The rotifer *Brachionus plicatilis* is a common brackish-water zooplankton, and one of the best-studied rotifer species. It is characterized by high growth rate, widespread distribution, ubiquity in aquatic systems, ease of culture, adequate size, ability to feed on a variety of feed types and a complex life cycle. It has been used extensively as a tool in aquaculture and ecotoxicology and constitutes a model organism in ecological and evolutionary studies. This paper explores other possible uses of this organism in various fields: environmental control of eutrophication and harmful algal blooms, containment of cholera, management of pollution and petroleum compounds, wastewater treatment, impact of climate change on biodiversity and transfer of useful substances.

Key words: pollution, eutrophication, climate change, disease treatment.

INTRODUCTION

Rotifers are a relatively small group of invertebrates consisting of about 2000 named species of unsegmented, bilaterally symmetrical pseudocoelomates (Wallace & Snell, 1991; Wallace *et al.*, 2006; Segers, 2007). They account for a large proportion of zooplankton diversity in freshwater and brackish environments, inhabiting practically any body of water, from a trickle on the rocks to ponds, streams, marshes and salt lakes (Pejler, 1995). Because of their small size (40 µm–2 mm), rotifers constitute only a relatively minor part (about 2.5%) of zooplankton biomass, but they are significant in continental aquatic systems. Their importance lies in their exceptionally high reproductive rates, which are faster than those of any metazoan (Bennett & Boraas, 1989). Because of their high reproductive rates they occasionally numerically dominate zooplankton communities (Wallace & Smith, 2009). Moreover, they have the ability to populate vacant niches rapidly and are quite efficient gra-

zers, making primary production (phytoplankton and bacteria) available to secondary consumers (e.g. other zooplankton species, fish fry). Their role in the production cycle is of considerable importance (Starkweather, 1987; Armengol *et al.*, 2001; Wallace & Smith, 2009).

Despite their minute size, female rotifers are anatomically complex (Wallace & Snell, 1991). Rotifer males are dwarf and have simplified anatomy with reduced functions (Epp & Lewis, 1979; Ricci & Melone, 1998). The development is eutelic (i.e. constant cell number after ontogenetic development) and growth to final size is accomplished by mere stretching of existing cells. Rotifers are characterized by two distinct features: a corona (a ciliated region at the anterior end), used for locomotion (i.e. swimming) and food gathering, and a specialized pharynx, the mastax, which serves as a jaw (Ruttner-Kolisko, 1974; Nogrady *et al.*, 1993).

The phylum Rotifera contains three classes. The largest class – monogononts (more than 1500 species) – reproduces by cyclical parthenogenesis, a life cycle which combines asexual and sexual reproduc-

* Corresponding author: tel.: +30 2810 337766, fax: +30 2810 337778, e-mail: vkostop@biol.uoa.gr

tion (Fig. 1). Typically, a rotifer population grows parthenogenetically (asexual proliferation), whereby a repeated number of generations of amictic (asexual) females produce mitotically diploid eggs. These eggs hatch into genetically identical amictic female offspring. Following certain environmental cues, such as population density and photoperiod (Carmona *et al.*, 1993; Gilbert, 2004; Snell *et al.*, 2006), amictic females produce mictic (sexual) female individuals as some fraction of their offspring. Mictic females meiotically give rise to haploid eggs. These eggs, if not fertilized, develop into haploid males. The latter can inseminate other mictic females, whose fertilized eggs will develop into diploid encysted embryos (resting eggs), which undergo diapause. Once produced, resting eggs sink and settle in the sediment. Resting eggs are resistant to harsh environmental conditions, such as drying or freezing, and may be dispersed over wide areas by the wind, water or migrating animals (Gil-

bert, 1974; Schröder, 2005). After an obligatory dormant period, and taken that conditions become favourable, resting eggs hatch into amictic females that enter into the asexual phase of the life cycle (Ruttner-Kolisko, 1974; Nogrady *et al.*, 1993; Wallace *et al.*, 2006). A fraction of the diapausing eggs do not hatch when the conditions are favorable, which results in a pool of diapausing eggs in the sediment, the so-called ‘egg bank’ (Marcus *et al.*, 1994; Hairston, 1996).

Cyclical parthenogenesis combines the advantages of rapid multiplication, when conditions are favourable to exploit resources (parthenogenesis), with long-term survival through resting egg production, when conditions deteriorate (sexual reproduction). Parthenogenesis eliminates the problem of mating encounters and the cost of producing males, allowing an asexual population to grow faster than a sexual one –intrinsic growth rate difference being dependent on the level of sexuality (Serra & Snell, 2009; Stelzer, 2011).

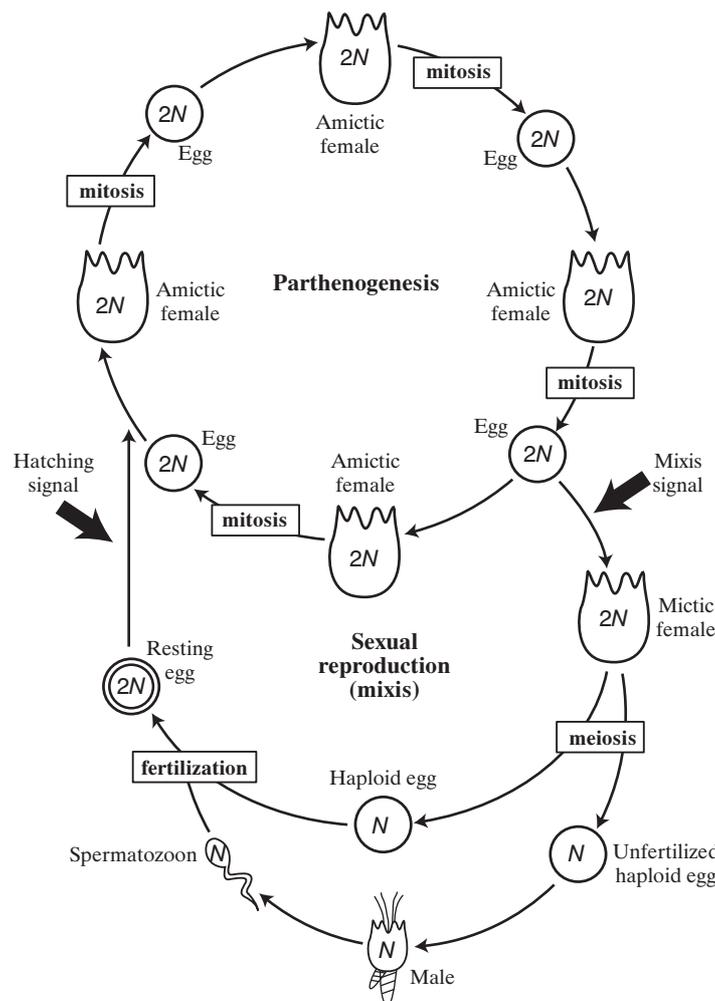


FIG. 1. Schematic representation of cyclical parthenogenesis in the rotifer *Brachionus plicatilis*.

Cyclical parthenogenesis serves to produce clones best adapted to prevailing conditions. These clones are theoretically capable of successfully exploiting the existing habitat. In that respect, population growth is not limited by initial sparseness and a single individual is theoretically capable of colonizing a new habitat (Gerritsen, 1980). Sexual reproduction on the other hand, produces genetic variation in offspring through the mechanism of sexual recombination. This results in higher rates of adaptation and inhibits the accumulation of deleterious mutations (e.g. West *et al.*, 1999). Moreover, the linkage between dormancy and sex allows long-term survival of rotifer populations (Carmona *et al.*, 2009; Serra & Snell, 2009). Sexual reproduction, through the production of resting eggs, offers environmental escape in space and time (Pourriot & Snell, 1983; Serra *et al.*, 2004).

One of the best known monogonont rotifers is *Brachionus plicatilis* (Müller, 1786). This rotifer has been extensively studied, owing to its use in aquaculture (Lubzens, 1987). Once considered a pest (“mizukawari” – Hirata, 1980), it now forms an indispensable element of hatcheries, where it is offered as first feed to fish larvae.

Brachionus plicatilis has been classified as an r-strategist (Walker, 1981; Miracle *et al.*, 1988), due to its small size, rapid growth and low C-value (i.e. DNA content), which has been estimated between 55 and 407 Mbp (Stelzer *et al.*, 2011). DNA content is highly correlated in eukaryotes with cell and nuclear volume, cell cycle length and minimum generation time (Cavalier-Smith, 1978). Based on the above, smaller genomes will result in more rapid mitotic division and cell cycles, conferring faster growth rates and earlier age at first reproduction. Such rapid development will eventually enhance the likelihood of contribution to the gene pool of the next generation when the environment is ephemeral (Wyngaard *et al.*, 2005).

According to the above, *B. plicatilis* is capable of quick colonization of a habitat, once appropriate conditions arise. It is a strategist of ephemeral or otherwise fluctuating habitats, such as temporary saline lakes and brackish coastal lagoons that often dry during the summer months (Ruttner-Kolisko, 1974; Walker, 1981; Gómez *et al.*, 1995). The occurrence of *B. plicatilis* in extreme environments points towards its remarkable tolerance to abiotic conditions (Epp & Winston, 1977; Walker, 1981; Esparcia *et al.*, 1989). It has been detected in all continents with the exception of the Antarctic (Segers, 2007). The widespread distribution of *B. plicatilis* suggests an efficient means of

dispersal via resting eggs (Walker, 1981; Gómez *et al.*, 2002).

In most of the recent literature, *B. plicatilis* was thought to be a single species, cosmopolitan and generalist. However, its revised taxonomical status has revealed an under-determined ancient cryptic species complex, comprising of at least 14 species/lineages (Gomez *et al.*, 2002; Suatoni *et al.*, 2006). Such ‘hidden’ diversity is expected to revolutionize the study of this taxa. For this reason, molecular tools, aiming to facilitate the identification of the species complex, are investigated (Papakostas *et al.*, 2005, 2006a; Doms *et al.*, 2007; Vasileiadou *et al.*, 2009). It has already been shown that the different species/lineages have a more restricted distribution and ecological range of tolerance than the complex as a whole (Ciros-Perez *et al.*, 2001; Ortells *et al.*, 2003). In nature, they have been shown to either coexist and/or succeed one another along the seasonal cycle (Serra *et al.*, 1998; Ortells *et al.*, 2003; Montero-Pau *et al.*, 2011). However, in aquaculture farms only a small fraction of the *B. plicatilis* genetic diversity is being exploited (Papakostas *et al.*, 2006b, 2009).

In nature, *B. plicatilis* feeds mainly on phytoplankton, although organic detritus and bacteria can also represent alternative feeding sources (Pourriot, 1977; Starkweather, 1980; Arndt, 1993). In hatcheries, *B. plicatilis* is also able to grow on formulated diets, prepared to fulfil the specific dietary requirements of fish larvae (Lubzens *et al.*, 2001).

Apart from aquaculture, *B. plicatilis* has been also used in basic research as a model organism. This is due to a number of characteristics, listed in Table 1. Population dynamics studies using the life-table approach have been numerous (Korstad *et al.*, 1989; Schmid-Araya, 1991; Serra *et al.*, 1994; Yoshinaga *et al.*, 2000). Rotifers were among the first organisms to be used in studies of biological aging (King, 1969; Enesco, 1993). It has been recently argued that *B. plicatilis* could be potentially rewarding for aging research (Austad, 2009). *Brachionus plicatilis* has been studied in terms of its biochemistry, morphology, physiology, as well as the molecular basis of aging (Luciani *et al.*, 1983; Carmona *et al.*, 1989; Yoshinaga *et al.*, 2003). Owing to its dual mode of reproduction, *B. plicatilis* has been used as a bio-model regarding the evolutionary significance of sex (e.g. Aparici *et al.*, 2002; Serra *et al.*, 2004; Carmona *et al.*, 2009). Its widespread distribution has facilitated studies on cryptic speciation (Gómez & Snell, 1996; Serra *et al.*,

TABLE 1. Characteristics of the rotifer *Brachionus plicatilis* that make it an attractive candidate as a tool in numerous research fields. References are for the listed characteristics

Characteristic	Field	References
Ubiquity in aquatic systems	Cryptic speciation, Molecular phylogenetics, Ecotoxicology	Koste & Shiel (1980), Miracle & Vicente (1983), Arndt (1988), Green & Mengestou (1991), Timms (1993), Turner (1993), Egborge (1994), Modenutti (1998), Zakaria <i>et al.</i> (2007)
High growth rate compared to other zooplankters	Aquaculture, Basic biological research	Allan (1976)
High ingestion rate	Aquaculture, Ecotoxicology	Navarro (1999)
Adequate size for: <ul style="list-style-type: none"> • feeding fish larvae • culture in small (μl) volumes 	Aquaculture, Basic biological research, Ecotoxicology	Lubzens <i>et al.</i> (2001)
Important role in energy flow and nutrient cycling	Ecology	Starkweather (1987), Armengol <i>et al.</i> (2001), Wallace & Smith (2009)
Ease of culture	Aquaculture, Basic biological research, Ecotoxicology	Hoff & Snell (1987)
Short generation time	Aquaculture, Basic biological research, Ecotoxicology	Korstad <i>et al.</i> (1989), Yoshinaga <i>et al.</i> (2003)
Ability to grow on a variety of food sources (phytoplankton, bacteria, inert food)	Aquaculture, Basic biological research	Starkweather (1980), Lubzens <i>et al.</i> (2001)
Use as a “living capsule”, transferring administered substances to recipient organism (predator)	Aquaculture	Lubzens <i>et al.</i> (2001)
Complex life cycle combining asexual and sexual reproduction, allowing for genetically identical individuals (clones) as well as the possibility of storage in the form of cysts, which can be readily available when needed	Basic biological research, Evolutionary ecology	Nogrady <i>et al.</i> (1993), Wallace & Snell (1991)
Resting egg production	Aquaculture, Ecology, Evolutionary ecology	Pourriot & Snell (1983)
Eutely	Biology of development and aging	Nogrady <i>et al.</i> (1993), Wallace (2002)
Transparency of body	Biology of development and aging	Wallace (2002)
Well-studied biology	All fields	Ricci <i>et al.</i> (2000)

1997) and molecular phylogenetics (Gómez *et al.*, 2002; Suatoni *et al.*, 2006; Mills *et al.*, 2007). Basic knowledge on genomics is just emerging (Suga *et al.*, 2007, 2008; Montero-Pau & Gómez, 2011). Rotifers have been also considered as good indicators in ecotoxicology (Sládeček, 1983); standard methods have been developed, that are rapid, sensitive, reliable, of good repeatability and cost-effectiveness (Snell & Persoone, 1989; Ferrando & Andrew-Moliner, 1992; Moffat & Snell, 1995; Snell & Janssen, 1995; Del-Valls *et al.*, 1996, 1997).

Recently, it has been proposed that *B. plicatilis* could be also used as model organism in evolutionary developmental biology (evo-devo) (Boell & Bucher, 2008). This particular field aims at reconstructing evolutionary relationships between animals going back to the origins of bilateral symmetry. The phylogenetic position of rotifers lies within the Lophotrochozoans, which belong to the protostome branch of Bilateria (Dunn *et al.*, 2008). Most protostome model systems belong to the Ecdysozoa branch, whereas Lophotrochozoans are underrepresented. In addition, there is overrepresentation of segmented *versus* non-segmented taxa (Boell & Bucher, 2008). *Brachionus plicatilis* could therefore represent a suitable non-segmented model organism, belonging to the Lophotrochozoans, for comparative analysis of gene expression.

It therefore becomes obvious that the rotifer *B. plicatilis* is a very useful, but still unexplored tool for numerous applications. Some of these are explored below.

POTENTIAL APPLICATIONS USING THE ROTIFER *BRACHIONUS PLICATILIS*

Environmental management of eutrophication

Although the definition of eutrophication is still on debate (Andersen *et al.*, 2006), Nixon (1995) gave a rather straightforward description: ‘an increase in the rate of supply of organic matter to an ecosystem’. Eutrophication has been considered one of the major threats to the health of marine ecosystems (e.g. Smith *et al.*, 2006). It is related to the input mainly of nitrogen and phosphorus and results in an increased growth of algae, with direct consequences on water quality. The latter have been well known and documented (Cloern, 2001).

The control of algal growth in lakes can be attained by bio-manipulation of food webs through processes such as zooplankton grazing (top-down control) of algal biomass (Moss *et al.*, 1994; Beklioglu, 1999; Schindler, 2006). To affect a dense phytoplankton bloom significantly, a given organism must satisfy several requirements: it must be abundant, it must coincide with algae both in space and time and it must be able to feed on them efficiently (Calbet, 2008). Rotifers have short developmental time, high filtration rate and can quickly reach high densities. In comparison to other organisms, they are particularly capable of locating and exploiting food patches until depletion (Ignoffo *et al.*, 2005). These characteristics make *B. plicatilis* a potentially successful candidate in the control of phytoplankton growth (eutrophication) in brackish and marine coastal ecosystems. Rotifers in general are considered good indicators of eutrophication (Sládeček, 1983; Park & Marshall, 2000; Turton & McAndrews, 2006; Zakaria *et al.*, 2007) and *B. plicatilis* in particular shows increased abundance when conditions become eutrophic in nature (Arndt, 1988; Gaudy *et al.*, 1995; Haberman & Sudzuki, 1998; Zakaria *et al.*, 2007). Therefore, introduction of rotifers into waters containing high concentrations of algae may increase grazing pressure, resulting in a reduction of abnormally high levels of phytoplankton.

In the specific case of Harmful Algal Blooms (HABs), rotifer short developmental times also contribute to the appearance of resistant clones, able to successfully graze upon such organisms (Calbet *et al.*, 2003). *Brachionus plicatilis* is able to feed on a variety of phytoplankton species, including blue-green algae (Snell *et al.*, 1983). However, as demonstrated by Buskey & Hyatt (1995), Turner & Tester (1997), Kim *et al.* (2000) and Wang *et al.* (2005), such interactions can be situation-specific. The dinoflagellate *Karenia mikimotoi* and the raphidophyte *Heterosigma akashiwo* were both toxic to the rotifer *B. plicatilis*, which showed distinct morphological changes and reduced swimming speed upon contact (Xie *et al.*, 2008; Zou *et al.*, 2010). On the other hand, successful biocontrol by this rotifer was observed with the estuarine dinoflagellate *Pfiesteria piscicida* and the dinoflagellate *Alexandrium tamarense* (Mallin *et al.*, 1995; Xie *et al.*, 2008). Nevertheless, it has not been tested yet whether the toxin remains viable in the gut of the rotifer after consumption, leading to bioaccumulation (Mallin *et al.*, 1995). The rotifer *B. plicatilis* can be therefore used as a sensitive indicator and possibly, as a biological control tool in HABs, depending on species.

Environmental management of cholera

Eutrophication and harmful algal blooms may also provide a reservoir for water-borne diseases, such as cholera (Epstein, 1993). *Vibrio cholerae*, organism responsible for this disease, shows enhanced survival and persistence when associated to algae and/or copepods, relative to the surrounding water. The latter organisms provide protection and nutrition to *V. cholerae*, especially under unfavorable conditions (Heidelberg et al., 2002; Lipp et al., 2002). Therefore, increased algal growth where *V. cholera* is present will facilitate spread of cholera. *Brachionus plicatilis* could prove useful in limiting the environmental dispersion of the disease. *Vibrio cholerae* is naturally present in warm, brackish waters (Lipp et al., 2002), where *B. plicatilis* is also encountered. Indeed, *Brachionus* species have been detected in areas where cholera is endemic (Tamplin et al., 1990). Freshwater rotifers have been shown to ingest protozoan parasites that are widely distributed in the aquatic environment (Fayer et al., 2000; Trout et al., 2002; Nowosad et al., 2007). It is not known whether *B. plicatilis* is also capable of retaining *V. cholerae*, although bacterivory by this rotifer is considered to be substantial (Turner & Tester, 1992). Still, indirect containment of *V. cholerae* through consumption of phytoplankton could be an alternative strategy.

Environmental management of pollution

In the wider context of disturbance, pollution represents another field where rotifers could play a role. A suitable indicator species should have certain attributes: it should be easily cultured in a small volume of water, preferably without the occurrence of sexual reproduction. In addition, the organism must react clearly and death must be unequivocal (Sládeček, 1983). Rotifers fulfill the abovementioned requirements. In nature, they are considered good indicators of water quality (Sládeček, 1983; Saksena, 1987). *Brachionus plicatilis* in particular has been used in ecotoxicological studies in the lab (Snell & Janssen, 1995), as well as indicator species in the field (Sharma, 1983).

To go a step further, from detection to control, organisms can be used to actually degrade or convert environmental contaminants to innocuous end products, a process known as bioremediation (Thassitou & Arvanitoyannis, 2001). Algae and/or plants have been used to successfully clean up hazardous waste (Gekeler et al., 1988; Ahner et al., 1995; Hitchcock et al., 2003; Yoshida et al., 2009). However, concerns arise

as to the potential adverse effects of breakdown/transformation products resulting from such processes (Hitchcock et al., 2003). In order to assess the impact of phytoremediation products to higher trophic levels, rotifers have been employed (Moreno-Garrido et al., 1999; Hitchcock et al., 2003; Rioboo et al., 2007). In general, phytoremediation end products had a negative influence of varying magnitude on rotifers most of the times. Recovery was observed when rotifers were returned to toxicant-free media (Rioboo et al., 2007) or supplied with high food concentrations (Luna-Andrade et al., 2002). Although algae have been shown to be more resistant to toxicants than rotifers (Luna-Andrade et al., 2002), observed changes are not necessarily conclusive. For example, *B. plicatilis* shows high tolerance to i) insecticides (Serrano et al., 1986; Snell & Persoone, 1989; Ferrando & Andreu-Moliner, 1991), ii) certain heavy metals (Persoone et al., 1989; Snell & Persoone, 1989; Snell et al., 1991) and iii) petroleum compounds (Snell et al., 1991; Ferrando & Andreu-Moliner, 1992). Under conditions of ample food and reduced competition, bioremediation using rotifers can be further reinforced to give optimal results (see Yasuno et al., 1993).

Environmental management of petroleum compounds

The tolerance of rotifers to petroleum compounds could prove useful in the control of oil spills, especially in enclosed habitats, which are more prone than the open ocean, due to reduced dilution capacity. Oil spills usually cause an upsurge of microbial and plant biomass, later to be followed by small zooplankton, particularly rotifers (Johansson et al., 1980; Davenport et al., 1982; Linden et al., 1987). *Brachionus plicatilis* has been shown to actively accumulate hydrocarbons; whether it is able to metabolize them has not been tested yet, but remains a possibility (Echeverria, 1980; Wolfe et al., 1998). Perhaps more worrying than episodic oil spills are the consequences arising from the continuous presence of oil products, such as tar balls, blobs of semi-solid oil, which are commonly encountered in enclosed seas associated with oil exploitation (Red Sea, Arabian Gulf, Mediterranean Sea) (Morris, 1974; Davenport et al., 1982; Hanna, 1983; Holdway, 1986; Price & Nelson-Smith, 1986). These balls are usually neutrally buoyant, may remain in the water column for long periods and eventually wash ashore coating shoreline sediment (Eagle et al., 1979; Sen Gupta et al., 1993). Zooplankton is able to graze upon particulate tar balls, providing in this way

a mechanism of rapid sedimentation to greater depths through faecal pellets (Sleeter & Butler, 1982). Rotifers, in particular, owing to their high ingestion rate, could prove useful in the abatement of tar balls.

Wastewater treatment

Wastewater treatment is another area where rotifers could play a leading role. Different systems are used worldwide for the treatment of wastewater, such as activated sludge, trickling filters and waste stabilization ponds. Each of these systems operates on the same fundamental biochemical principles (bacteria are primarily used in pollutant removal) and differs on the method of oxygen transfer (activated sludge utilizes compressed air, trickling filters obtain their oxygen by diffusion from the air and ponds use algae) and source of wastes (activated sludge and trickling filters are used in industrial wastes, whereas waste ponds are used for domestic and agro-industrial wastewaters) (McKinney, 1957; Patil *et al.*, 1993; Roche, 1995; El-Deeb Ghazy *et al.*, 2008). Freshwater rotifers are encountered in activated sludge systems (Poole, 1984) and waste stabilization ponds (Patil *et al.*, 1993; Roche, 1995) and are (in a different way) instrumental in the functioning of both systems.

In the case of activated sludge systems, rotifers can consume filamentous bacteria that create foaming and bulking, as well as sludge particles themselves. In that way, they improve the settling properties and clarity of sludge, as well as reduce biomass production. Disposal of excess sludge is considered a major bottleneck of wastewater treatment and rotifers could therefore prove to be an economical and sustainable solution to this problem (Lee & Welander, 1996; Lapinski & Tunnacliffe, 2003; Fialkowska & Pajdak-Stos, 2008). On the other hand, in waste stabilization ponds, freshwater rotifers play an important role in the purification of wastewater through the consumption of dispersed or coagulated bacteria, organic matter and phytoplankton (Patil *et al.*, 1993; Zhao & Wang, 1996). It has been proposed that, owing to the use of both algae and rotifers in aquaculture, the latter two organisms could be produced using wastewater. This could become a low-cost alternative to expensive phytoplankton and rotifer culture and a way to recycle nutrients. However, nutritional adequacy, organic overloading and presence of pathogens will have to be investigated (Uhlmann, 1980; Groeneweg & Schluter, 1981; Roche, 1995; Cauchie *et al.*, 2000; Sarma *et al.*, 2003).

Tracking climate change

Climate change is now recognized as one of the major environmental problems facing the earth. The burning of fossil fuels and deforestation have caused an increase in the concentrations of heat-trapping “greenhouse gases”, such as carbon dioxide (CO₂) and methane (CH₄) in the atmosphere, resulting in global warming (Chapin *et al.*, 2000). Over the past 100 years, the Earth’s climate is warmed by approximately 0.6 °C (Walther *et al.*, 2002). These changes are expected to trigger phenomena like sea level rise, more frequent and intense extreme weather events and ocean acidification, to mention a few. There is growing evidence that climate change will contribute to shifts in the geographic range of species, alterations in the timing of important life-history events, disruption of food webs (McCarthy, 2001; Root *et al.*, 2003; Richardson, 2008), even accelerated species losses (Wrona *et al.*, 2006). However, large uncertainties remain in projecting species and system-specific responses. In addition, other stresses, in particular habitat destruction, but also increased susceptibility to pathogens and pests, could further exacerbate the effects of climate change on organisms (Harvell *et al.*, 1999; McCarthy, 2001; Root *et al.*, 2003).

Marine pelagic communities are said to be affected to a greater extent, compared to terrestrial communities, because of the temperature influence on water column stability (Edwards & Richardson, 2004; Richardson, 2008) and the important role of the ocean in the uptake of anthropogenic CO₂ (Hays *et al.*, 2005; Fabry *et al.*, 2008). Plankton in particular is considered a good indicator of climate change (Hays *et al.*, 2005; Richardson, 2008): (1) it is sensitive to temperature changes as it is composed of ectothermic organisms, (2) it is not commercially exploited, (3) it is short-lived, so past populations do not exert an influence on present ones, (4) it is free floating, so it can show changes in its distribution in response to climate change and (5) it is more sensitive than environmental variables themselves, as it can amplify subtle perturbations. It is therefore important to test the projected effects of global warming using a test organism from the plankton community. Copepods have been extensively studied, owing to their importance in the open ocean (Richardson, 2008). However, the open ocean, due to its size and permanence, has the capacity to dampen out to a certain extent climatic fluctuations. Ephemeral and extreme habitats are instead more vulnerable to perturbations (Gaudy *et al.*, 1995)

and should be more sensitive to climate change. They could provide an early indication of the biological impact of shifting climate. The importance of such habitats also lies in their ecological value, as they are considered biodiversity hotspots (Walsh *et al.*, 2008; Angeler *et al.*, 2010). *Brachionus plicatilis* is an inhabitant of such habitats and could therefore serve as an indicator r-type organism of climate change.

Can *B. plicatilis* track the effects of climate change? Climate change is mainly manifested by a rise in temperature, a decrease in pH, as a consequence of acidification (Fabry *et al.*, 2008) and drying of ephemeral habitats. Temperature has a direct effect on organisms. Rotifers are ectothermic organisms, so their metabolism is directly exposed to the temperature of their environment (Stelzer, 1998). Consequently, temperature is the most important factor shaping the population dynamics of rotifers (Galkovskaja, 1987; Arndt, 1988; Miracle & Serra, 1989; Gaudy *et al.*, 1995). This is manifested by the seasonal component that characterizes the occurrence of *B. plicatilis* in nature (Walker, 1981; Miracle *et al.*, 1987; Arndt, 1988; Haberman & Sudzuki, 1998; Modenutti, 1998; Jellison *et al.*, 2001; Zakaria *et al.*, 2007), which is expected to be affected by climate change. On the other hand, temperature affects critical life cycle events such as hatching of resting eggs (Pourriot & Snell, 1983), with direct consequences on the structuring of food webs. It has been shown that differential hatching of resting eggs due to rising temperatures resulted in a selective advantage of rotifers over cladocerans in freshwater ecosystems (Winder & Schindler, 2004; Dupuis & Hann, 2009). Therefore, *B. plicatilis* offers the opportunity to study the direct as well as the indirect effects of changing temperature.

Low pH values adversely affect survival, longevity, reproduction, Na⁺ flux, growth rate, feeding and respiration in zooplankton (Locke, 1991). Freshwater rotifers have been shown to dominate zooplankton communities in highly acidic lakes, due to their broad pH tolerance (Berzins & Pejler, 1987; Frost *et al.*, 1998; Deneke, 2000). *Brachionus plicatilis* in particular has not received much attention as to its pH tolerance, although reported values cover the near neutral-alkaline range (6.5-9.8) (Walker, 1981; Turner, 1993; Haberman & Sudzuki, 1998; Modenutti, 1998; Ortells *et al.*, 2000). *Brachionus plicatilis* is an inhabitant of alkaline environments, so there is no available information as to how this species will respond to acidification. In the absence of field data, tolerance of *B. plicatilis* to low pH could be experimentally mea-

sured, using indices such as swimming speed, respiration and filtering rate (Epp & Winston, 1978; Locke, 1991).

Laboratory-derived data can be used to explain observed distributions, but predictions cannot be solely based on physiological rates. Other factors should be taken into account, namely the overall characteristics of the changing environment or habitat that the organism has moved to (Feder, 2010). It is therefore crucial to follow *B. plicatilis* distribution in the field and to compare it with past records, in order to be able to discern the influence of climate change. Being a well-studied species, it is possible to find long-term studies on the distribution of *B. plicatilis* (Sharma, 1983; De Ridder, 1987). However, due to its recently revised taxonomic status, some data on past distributions could correspond to other species of the complex. To go further back in time, the resting egg bank can provide a snapshot from the past (Montero-Pau *et al.*, 2011).

Resting egg banks are formed and replenished every time a population appears in the water column and completes one “growing cycle”, usually on a yearly basis. The *B. plicatilis* is an ancient species complex (Gómez *et al.*, 2002; Derry *et al.*, 2003; Suatoni *et al.*, 2006), and, over the years, its occurrence has left its mark in the sediments (Pourriot & Snell, 1983; Gómez & Carvalho, 2000; Ortells *et al.*, 2000; García-Roger *et al.*, 2006a). Hatching of resting eggs is feasible after the lapse of considerable time spans (Marcus *et al.*, 1994; Kotani *et al.*, 2001; García-Roger *et al.*, 2006b). So, the accumulated biotic diversity stored in resting egg banks, can serve as an indication of past populations/climates, which can be compared to present ones (Montero-Pau *et al.*, 2011).

Last but not least, is the threat of extinction, stemming from potential drying of ephemeral habitats, like the ones *B. plicatilis* inhabits. Although resting egg banks have the capacity to buffer transient environmental perturbations (Hairston, 1996; Serra *et al.*, 2004), permanent changes cannot be overcome. A rotifer population experiencing three catastrophic crashes per year is certain to go extinct within 100 years (Snell & Serra, 2000). In addition, sexual reproduction, which ensures resting egg production and long-term survival, could be more susceptible to environmental change than parthenogenesis, due to its increased complexity. Sexual reproduction needs a longer time to complete, is more resource-demanding and is more sensitive to external influences (Snell & Boyer, 1988; Snell & Carmona, 1995; Serra *et al.*,

2004), in part due to its reliance on chemical communication (Snell *et al.*, 2006).

Transfer of useful substances

The rotifer *B. plicatilis* is a high-value, but nutritionally inadequate, prey for fish larvae, due to its lack of essential HUFAs (Highly Unsaturated Fatty Acids). This is why enrichment protocols have been devised that allow the transfer of required substances, mainly HUFAs, to fish larvae (Rainuzzo *et al.*, 1994a; Rodriguez *et al.*, 1998; Castell *et al.*, 2003). Transfer of HUFAs via rotifers has been shown to improve growth, survival and total length in gilthead seabream larvae (Rodriguez *et al.*, 1994, 1998), pigmentation in turbot larvae (Rainuzzo *et al.*, 1994b), survival and incidence of deformities in milkfish (Gapasin & Duray, 2001), size and survival in yellowtail flounder (Copeman *et al.*, 2002), among others. The transfer of vitamins and therapeutics has been also realized (Verpraet *et al.*, 1992; Merchie *et al.*, 1995; Fernandez *et al.*, 2008; Roiha *et al.*, 2011). Vitamin C significantly improved stress resistance in European sea bass, whereas vitamin A has been implicated in gilthead sea bream skeletogenesis. In accordance with the present use of rotifers in aquaculture, *B. plicatilis* can be used as a 'transfer capsule' of desirable substances to target organisms.

Interest has also turned towards the possible influence of bacteria on disease resistance. Techniques have been developed that allow the transfer of beneficial bacteria (probiotics), as well as immunostimulants, to fish larvae, through the rotifer *B. plicatilis* (Skjermo & Vadstein, 1999; Makridis *et al.*, 2000; Martínez-Díaz *et al.*, 2003; Pintado *et al.*, 2010). For example, probiotics have been shown to improve survival rate in turbot larvae challenged with *Vibrio* (53% survival rate *versus* 8% for the control group without probiotics as reported by Gatesoupe, 1994) (Planas *et al.*, 2006), survival (13-105% higher compared to control) and specific growth rate (2-9% higher compared to control) in gilthead sea bream larvae and fry (Carnevali *et al.*, 2004; Suzer *et al.*, 2008), body weight (81% with respect to control) and tolerance to captive rearing conditions in European sea bass juveniles (Carnevali *et al.*, 2006). A promising area of developing research focuses on axenic rotifers (gnotobiotic), which can be used as an experimental *in vivo* system for the study of host-microbe interactions, nutritional functions in aquatic food chains, even evaluation of new treatments of disease

control (Tinh *et al.*, 2006, 2007; Marques *et al.*, 2006). The abovementioned techniques that have been developed for aquaculture could also find applications in other fields. In this procedure, the rotifer *B. plicatilis* could play a leading role.

CONCLUSIONS

As proposed in the present paper, the rotifer *Brachionus plicatilis* could serve a number of possible applications. This is why initiatives should be taken as to the study, buffering capacity and preservation of this species complex already having many applications (aquaculture, water quality indicator, model organism in basic research). The creation of a rotifer bank (i.e. *ex situ* storage of rotifers and/or their resting eggs) could serve such a purpose.

The proposed rotifer bank would constitute of rotifer strains originating from the field and mass production (hatcheries). These rotifer strains would be characterized as to their taxonomic status and biological characteristics. All this information could be used in favor of mass production: hatcheries could be supplied with rotifers that best fit their needs. In this way, the rotifer bank would improve the operation and production of hatcheries. On a second level, it would contribute to the conservation of biodiversity and serve the advancement of science. Numerous applications are waiting to be realized in the future.

REFERENCES

- Allan JD, 1976. Life history patterns in zooplankton. *American Naturalist*, 110: 165-180.
- Andersen JH, Schlüter L, Ærtebjerg G, 2006. Coastal eutrophication: recent developments in definitions and implications for monitoring strategies. *Journal of Plankton Research*, 28: 621-628.
- Angeler DG, Alvarez-Cobelas M, Sanchez-Carrillo S, 2010. Evaluating environmental conditions of a temporary pond complex using rotifer emergence from dry soils. *Ecological Indicators*, 10: 545-549.
- Ahner BA, Kong S, Morel FMM, 1995. Phytochelatin production in marine algae. 1. An interspecies comparison. *Limnology and Oceanography*, 40: 649-657.
- Aparici E, Carmona MJ, Serra M, 2002. Evidence for an even sex allocation in haplodiploid cyclical parthenogens. *Journal of Evolutionary Biology*, 15: 65-73.
- Armengol X, Boronat L, Camacho A, Wurtsbaugh WA, 2001. Grazing by a dominant rotifer *Conochilus unicornis* Rousset in a mountain lake: *in situ* measurements with synthetic microspheres. *Hydrobiologia*, 446/447: 107-114.

- Arndt H, 1988. Dynamics and production of a natural population of *Brachionus plicatilis* (Rotatoria, Monogononta) in a eutrophicated inner coastal water of the Baltic. *Kieler Meeresforschungen Sonderheft*, 6: 147-153.
- Arndt H, 1993. Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) – a review. *Hydrobiologia*, 255/256: 231-246.
- Austad SN, 2009. Is there a role for new invertebrate models for aging research? *Journal of Gerontology: Biological Sciences*, 64A: 192-194.
- Beklioglu M, 1999. A review on the control of eutrophication in deep and shallow lakes. *Turkish Journal of Zoology*, 23: 327-336.
- Bennett WN, Boraas ME, 1989. A demographic profile of the fastest growing metazoan: a strain of *Brachionus calyciflorus* (Rotifera). *Oikos*, 55: 365-369.
- Berzins B, Pejler B, 1987. Rotifer occurrence in relation to pH. *Hydrobiologia*, 147: 107-116.
- Boell LA, Bucher G, 2008. Whole-mount *in situ* hybridization in the rotifer *Brachionus plicatilis* representing a basal branch of lophotrochozoans. *Development Genes and Evolution*, 218: 445-451.
- Buskey EJ, Hyatt CJ, 1995. Effects of the Texas (USA) 'brown tide' alga on planktonic grazers. *Marine Ecology Progress Series*, 126: 285-292.
- Calbet A, 2008. The trophic roles of microzooplankton in marine systems. *ICES Journal of Marine Science*, 65: 325-331.
- Calbet A, Vaqué D, Felipe J, Vila M, Montserrat Sala M, Alcaraz M, Estrada M, 2003. Relative grazing impact of microzooplankton and mesozooplankton on a bloom of the toxic dinoflagellate *Alexandrium minutum*. *Marine Ecology Progress Series*, 259: 303-309.
- Carmona MJ, Serra M, Miracle MR, 1989. Protein patterns in rotifers: the timing of aging. *Hydrobiologia*, 186/187: 325-330.
- Carmona MJ, Serra M, Miracle MR, 1993. Relationships between mixis in *Brachionus plicatilis* and preconditioning of culture medium by crowding. *Hydrobiologia*, 256/257: 145-152.
- Carmona MJ, Dimas-Flores N, García-Roger EM, Serra M, 2009. Selection of low investment in sex in a cyclically parthenogenetic rotifer. *Journal of Evolutionary Biology*, 22: 1975-1983.
- Carnevali O, Zamponi MC, Sulpizio R, Rollo A, Nardi M, Orpianesi C, Silvi S, Caggiano M, Polzonetti AM, Cresci A, 2004. Administration of probiotic strain to improve sea bream wellness during development. *Aquaculture International*, 12: 377-386.
- Carnevali O, de Vivo L, Sulpizio R, Gioacchini G, Olivotto I, Silvi S, Cresci A, 2006. Growth improvement by probiotic in European sea bass juveniles (*Dicentrarchus labrax*, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquaculture*, 258: 430-438.
- Castell J, Blair T, Neil S, Howes K, Mercer S, Reid J, Young-Lai W, Gullison B, Dhert P, Sorgeloos P, 2003. The effect of different HUFA enrichment emulsions on the nutritional value of rotifers (*Brachionus plicatilis*) fed to larval haddock (*Melanogrammus aeglefinus*). *Aquaculture International*, 11: 109-117.
- Cauchie H-M, Hoffmann L, Thomé J-P, 2000. Metazooplankton dynamics and secondary production of *Daphnia magna* (Crustacea) in an aerated waste stabilization pond. *Journal of Plankton Research*, 22: 2263-2287.
- Cavalier-Smith T, 1978. Nuclear volume control by nucleoskeletal DNA, selection of cell volume and cell growth rate, and the solution of the DNA C-value paradox. *Journal of Cell Science*, 34: 247-278.
- Chapin FS III, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL, Hooper DU, Lavorel S, Sala OE, Hobbie SE, et al., 2000. Consequences of changing biodiversity. *Nature*, 405: 234-242.
- Ciros-Pérez J, Gómez A, Serra M, 2001. On the taxonomy of three sympatric sibling species of the *Brachionus plicatilis* (Rotifera) complex from Spain, with the description of *B. ibericus* n. sp. *Journal of Plankton Research*, 23: 1311-1328.
- Cloern JE, 2001. Our evolving conceptual model of the coastal eutrophication problem. *Marine Ecology Progress Series*, 210: 223-253.
- Copeman LA, Parrish CC, Brown JA, Harel M, 2002. Effects of docosahexaenoic, eicosapentaenoic, and arachidonic acids on the early growth, survival, lipid composition and pigmentation of yellowtail flounder (*Limanda ferruginea*): a live food enrichment experiment. *Aquaculture*, 210: 285-304.
- Davenport J, Angel MV, Gray JS, Crisp DJ, Davies JM, 1982. Oil and planktonic ecosystems [and discussion]. *Philosophical Transactions of the Royal Society of London Series B*, 297: 369-384.
- De Ridder M, 1987. Distribution of rotifers in African fresh and inland saline waters. *Hydrobiologia*, 147: 9-14.
- DelValls TA, Lubián LM, González del Valle M, Forja JM, 1996. Evaluating decline parameters of rotifer *Brachionus plicatilis* populations as an interstitial water toxicity bioassay. *Hydrobiologia*, 341: 159-167.
- DelValls TA, Lubián LM, Forja JM, Gomez-Parra A, 1997. Comparative ecotoxicity of interstitial waters in littoral ecosystems using Microtox® and the rotifer *Brachionus plicatilis*. *Environmental Toxicology and Chemistry*, 16: 2323-2332.
- Deneke R, 2000. Review of rotifers and crustaceans in highly acidic environments of pH values ≤ 3 . *Hydrobiologia*, 433: 167-172.
- Derry AM, Hebert PDN, Prepas EE, 2003. Evolution of rotifers in saline and subsaline lakes: A molecular phylogenetic approach. *Limnology and Oceanography*, 48: 675-685.
- Dooms S, Papakostas S, Hoffman S, Delbare D, Dierckens

- K, Triantafyllidis A, De Wolf T, Vadstein O, Abatzopoulos TJ, Sorgeloos P, Bossier P, 2007. Denaturing Gradient Gel Electrophoresis (DGGE) as a tool for the characterization of *Brachionus* sp. strains. *Aquaculture*, 262: 29-40.
- Dunn CS, Hejnal A, Matus DQ, Pang K, Browne WE, Smith SA, Seaver E, Rouse GW, Obst M, Edgecombe G, et al., 2008. Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature*, 452: 745-749.
- Dupuis AP, Hann BJ, 2009. Climate change, diapause termination and zooplankton population dynamics: an experimental and modelling approach. *Freshwater Biology*, 54: 221-235.
- Eagle GA, Green A, Williams J, 1979. Tar ball concentrations in the ocean around the Cape of Good Hope before and after a major oil spill. *Marine Pollution Bulletin*, 10: 321-325.
- Echeverria T, 1980. Accumulation of ¹⁴C labeled benzene and related compounds in the rotifer *Brachionus plicatilis* from seawater. *Canadian Journal of Fisheries and Aquatic Sciences*, 37: 738-741.
- Edwards M, Richardson AJ, 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, 430: 881-884.
- Egborge ABM, 1994. Salinity and the distribution of rotifers in the Lagos Harbour-Badagry Creek system, Nigeria. *Hydrobiologia*, 272: 95-104.
- El-Deeb Ghazy MM, El-Senousy WM, Abdel-Aatty AM, Kamel M, 2008. Performance evaluation of a waste stabilization pond in a rural area in Egypt. *American Journal of Environmental Sciences*, 4: 316-325.
- Enesco HE, 1993. Rotifers in aging research: use of rotifers to test various theories of aging. *Hydrobiologia*, 255/256: 59-70.
- Epp RW, Winston PW, 1977. Osmotic regulation in the brackish-water rotifer *Brachionus plicatilis* (Müller). *Journal of Experimental Biology*, 68: 151-156.
- Epp RW, Winston PW, 1978. The effects of salinity and pH on the activity and oxygen consumption of *Brachionus plicatilis* (Rotatoria). *Comparative Biochemistry and Physiology*, 59A: 9-12.
- Epp RW, Lewis WM Jr, 1979. Sexual dimorphism in *Brachionus plicatilis* (Rotifera): evolutionary and adaptive significance. *Evolution*, 33: 919-928.
- Epstein PR, 1993. Algal blooms in the spread and persistence of cholera. *BioSystems*, 31: 209-221.
- Esparcia A, Miracle MR, Serra M, 1989. *Brachionus plicatilis* tolerance to low oxygen concentrations. *Hydrobiologia*, 186/187: 331-337.
- Fabry VJ, Seibel BA, Feely RA, Orr JC, 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science*, 65: 414-432.
- Fayer R, Trout JM, Walsh E, Cole R, 2000. Rotifers ingest oocysts of *Cryptosporidium parvum*. *Journal of Eukaryotic Microbiology*, 47: 161-163.
- Feder ME, 2010. Physiology and global climate change. *Annual Review of Physiology*, 72: 123-125.
- Fernández I, Hontoria F, Ortiz-Delgado JB, Kotzamanis Y, Estévez A, Zambonino-Infante JL, Gisbert E, 2008. Larval performance and skeletal deformities in farmed gilthead sea bream (*Sparus aurata*) fed with graded levels of Vitamin A enriched rotifers (*Brachionus plicatilis*). *Aquaculture*, 283: 102-115.
- Ferrando MD, Andreu-Moliner E, 1991. Acute lethal toxicity of some pesticides to *Brachionus calyciflorus* and *Brachionus plicatilis*. *Bulletin of Environmental Contamination and Toxicology*, 47: 479-484.
- Ferrando MD, Andreu-Moliner E, 1992. Acute toxicity of toluene, hexane, xylene, and benzene to the rotifers *Brachionus calyciflorus* and *Brachionus plicatilis*. *Bulletin of Environmental Contamination and Toxicology*, 49: 266-271.
- Fialkowska E, Pajdak-Stós A, 2008. The role of *Lecane* rotifers in activated sludge bulking control. *Water Research*, 42: 2483-2490.
- Frost TM, Montz PK, Gonzalez MJ, Sanderson BL, Arnott SE, 1998. Rotifer responses to increased acidity: long-term patterns during the experimental manipulation of Little Rock Lake. *Hydrobiologia*, 387/388: 141-152.
- Galkovskaja GA, 1987. Planktonic rotifers and temperature. *Hydrobiologia*, 147: 307-317.
- Gapasin RSJ, Duray MN, 2001. Effects of DHA-enriched live food on growth, survival and incidence of opercular deformities in milkfish (*Chanos chanos*). *Aquaculture*, 193: 49-63.
- García-Roger EM, Carmona MJ, Serra M, 2006a. Patterns in rotifer diapausing egg banks: density and viability. *Journal of Experimental Marine Biology and Ecology*, 336: 198-210.
- García-Roger EM, Carmona MJ, Serra M, 2006b. Hatching and viability of rotifer diapausing eggs collected from pond sediments. *Freshwater Biology*, 51: 1351-1358.
- Gatesoupe FJ, 1994. Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic vibrio. *Aquatic Living Resources*, 7: 277-282.
- Gaudy R, Verriopoulos G, Cervetto G, 1995. Space and time distribution of zooplankton in a Mediterranean lagoon (Etang de Berre). *Hydrobiologia*, 300/301: 219-236.
- Gekeler W, Grill E, Winnacker E-L, Zenk MH, 1988. Algae sequester heavy metals via synthesis of phytochelatin complexes. *Archives of Microbiology*, 150: 197-202.
- Gerritsen J, 1980. Sex and parthenogenesis in sparse populations. *American Naturalist*, 115: 718-742.
- Gilbert JJ, 1974. Dormancy in rotifers. *Transactions of the American Microscopical Society*, 93: 490-513.
- Gilbert JJ, 2004. Population density, sexual reproduction and diapause in monogonont rotifers: new data for *Brachionus* and a review. *Journal of Limnology*, 63: 32-36.
- Gómez A, Snell TW, 1996. Sibling species and cryptic spe-

- ciation in the *Brachionus plicatilis* species complex (Rotifera). *Journal of Evolutionary Biology*, 9: 953-964.
- Gómez A, Carvalho GR, 2000. Sex, parthenogenesis and genetic structure of rotifers: microsatellite analysis of contemporary and resting egg bank populations. *Molecular Ecology*, 9: 203-214.
- Gómez A, Temprano M, Serra M, 1995. Ecological genetics of a cyclical parthenogen in temporary habitats. *Journal of Evolutionary Biology*, 8: 601-622.
- Gómez A, Serra M, Carvalho GR, Lunt DH, 2002. Speciation in ancient cryptic species complexes: evidence from the molecular phylogeny of *Brachionus plicatilis* (Rotifera). *Evolution*, 56: 1431-1444.
- Green J, Mengestou S, 1991. Specific diversity and community structure of Rotifera in a salinity series of Ethiopian inland waters. *Hydrobiologia*, 209: 95-106.
- Groeneweg J, Schluter M, 1981. Mass production of freshwater rotifers on liquid wastes II. Mass production of *Brachionus rubens* Ehrenberg 1838 in the effluent of high-rate algal ponds used for the treatment of piggery waste. *Aquaculture*, 25: 25-33.
- Haberman J, Sudzuki M, 1998. Some notes on *Brachionus rotundiformis* (Tschugunoff) in Lake Palaeostomi. *Hydrobiologia*, 387/388: 333-340.
- Hairston NGJr, 1996. Zooplankton egg banks as biotic reservoirs in changing environments. *Limnology and Oceanography*, 41: 1087-1092.
- Hanna RGM, 1983. Oil pollution on the Egyptian Red Sea coast. *Marine Pollution Bulletin*, 14: 268-271.
- Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, Grimes DJ, Hofmann EE, Lipp EK, Osterhaus ADME, Overstreet RM, et al., 1999. Emerging marine diseases – Climate links and anthropogenic factors. *Science*, 285: 1505-1510.
- Hays GC, Richardson AJ, Robinson C, 2005. Climate change and marine plankton. *Trends in Ecology and Evolution*, 20: 337-344.
- Heidelberg JF, Heidelberg KB, Colwell RR, 2002. Bacteria of the γ -subclass *Proteobacteria* associated with zooplankton in Chesapeake Bay. *Applied and Environmental Microbiology*, 68: 5498-5507.
- Hirata H, 1980. Culture methods of the marine rotifer, *Brachionus plicatilis*. *Mini Review and Data File of Fisheries Research*, 1: 27-46.
- Hitchcock DR, McCutcheon SC, Smith MC, 2003. Using rotifer population demographic parameters to assess impacts on the degradation products from trinitrotoluene phytoremediation. *Ecotoxicology and Environmental Safety*, 55: 143-151.
- Hoff FH, Snell TW, 1987. *Plankton culture manual*. 3rd edition. Florida Aquafarms Inc., Dade City, FL.
- Holdway P, 1986. A circumnavigational survey of marine tar. *Marine Pollution Bulletin*, 17: 374-377.
- Ignoffo TR, Bollens SM, Bochdansky AB, 2005. The effects of thin layers on the vertical distribution of the rotifer, *Brachionus plicatilis*. *Journal of Experimental Marine Biology and Ecology*, 316: 167-181.
- Jellison R, Adams H, Melack JM, 2001. Re-appearance of rotifers in hypersaline Mono Lake, California, during a period of rising lake levels and decreasing salinity. *Hydrobiologia*, 466: 39-43.
- Johansson S, Larsson U, Boehm P, 1980. The Tsesis oil spill – Impact on the pelagic ecosystem. *Marine Pollution Bulletin*, 11: 284-293.
- Kim D, Sato Y, Oda T, Muramatsu T, Matsuyama Y, Honjo T, 2000. Specific toxic effect of dinoflagellate *Heterocapsa circularisquama* on the rotifer *Brachionus plicatilis*. *Bioscience, Biotechnology and Biochemistry*, 64: 2719-2722.
- King CE, 1969. Experimental studies of ageing in rotifers. *Experimental Gerontology*, 4: 63-79.
- Korstad J, Olsen Y, Vadstein O, 1989. Life history characteristics of *Brachionus plicatilis* (rotifera) fed different algae. *Hydrobiologia*, 186/187: 43-50.
- Koste W, Shiel RJ, 1980. Preliminary remarks on the characteristics of the rotifer fauna of Australia (Notogaea). *Hydrobiologia*, 73: 221-227.
- Kotani T, Ozaki M, Matsuoka K, Snell TW, Hagiwara A, 2001. Reproductive isolation among geographically and temporally isolated marine *Brachionus* strains. *Hydrobiologia*, 446/447: 283-290.
- Lapinski J, Tunnacliffe A, 2003. Reduction of suspended biomass in municipal wastewater using bdelloid rotifers. *Water Research*, 37: 2027-2034.
- Lee NM, Welander T, 1996. Use of protozoa and metazoa for decreasing sludge production in aerobic wastewater treatment. *Biotechnology Letters*, 18: 429-434.
- Linden O, Rosemarin A, Lindskog A, Hoglund C, Johansson S, 1987. Effects of oil and oil dispersant on an enclosed marine ecosystem. *Environmental Science and Technology*, 21: 374-382.
- Lipp EK, Huq A, Colwell RR, 2002. Effects of global climate on infectious disease: the cholera model. *Clinical Microbiology Reviews*, 15: 757-770.
- Locke A, 1991. Zooplankton responses to acidification: a review of laboratory bioassays. *Water, Air, and Soil Pollution*, 60: 135-148.
- Lubzens E, 1987. Raising rotifer for use in aquaculture. *Hydrobiologia*, 147: 245-255.
- Lubzens E, Zmora O, Barr Y, 2001. Biotechnology and aquaculture of rotifers. *Hydrobiologia*, 446/447: 337-353.
- Luciani A, Chasse J-L, Clement P, 1983. Aging in *Brachionus plicatilis*: the evolution of swimming as a function of age at two different calcium concentrations. *Hydrobiologia*, 104: 141-146.
- Luna-Andrade A, Aguilar-Duran R, Nandini S, Sarma SSS, 2002. Combined effects of copper and microalgal (*Tetraselmis suecica*) concentrations on the population growth of *Brachionus plicatilis* Muller (Rotifera). *Water, Air, and Soil Pollution*, 141: 143-153.

- Makridis P, Fjellheim AJ, Skjermo J, Vadstein O, 2000. Control of the bacterial flora of *Brachionus plicatilis* and *Artemia franciscana* by incubation in bacterial suspensions. *Aquaculture*, 185: 207-218.
- Mallin MA, Burkholder JM, Larsen LM, Glasgow HB Jr, 1995. Response of two zooplankton grazers to an ichthyotoxic estuarine dinoflagellate. *Journal of Plankton Research*, 17: 351-363.
- Marcus NH, Lutz R, Burnett W, Cable P, 1994. Age, viability, and vertical distribution of zooplankton resting eggs from an anoxic basin: evidence of an egg bank. *Limnology and Oceanography*, 39: 154-158.
- Marques A, Ollevier F, Verstraete W, Sorgeloos P, Bossier P, 2006. Gnotobiotically grown aquatic animals: opportunities to investigate host-microbe interactions. *Journal of Applied Microbiology*, 100: 903-918.
- Martínez-Díaz SF, Álvarez-González CA, Legorreta MM, Vásquez-Juárez R, Barrios-González J, 2003. Elimination of the associated microbial community and bioencapsulation of bacteria in the rotifer *Brachionus plicatilis*. *Aquaculture International*, 11: 95-108.
- McCarthy JP, 2001. Ecological consequences of recent climate change. *Conservation Biology*, 15: 320-331.
- McKinney RE, 1957. Activity of microorganisms in organic waste disposal. II. Aerobic processes. *Applied and Environmental Microbiology*, 5: 167-174.
- Merchie G, Lavens P, Dhert P, Pector R, Mai Soni AF, Nelis H, Ollevier F, De Leenheer A, Sorgeloos P, 1995. Live food mediated vitamin C transfer to *Dicentrarchus labrax* and *Clarias gariepinus*. *Journal of Applied Ichthyology*, 11: 336-341.
- Mills S, Lunt DH, Gómez A, 2007. Global isolation by distance despite strong regional phylogeography in a small metazoan. *BMC Evolutionary Biology*, 7: 225-235.
- Miracle MR, Vicente E, 1983. Vertical distribution and rotifer concentrations in the chemocline of meromictic lakes. *Hydrobiologia*, 104: 259-267.
- Miracle MR, Serra M, 1989. Salinity and temperature influence in rotifer life history characteristics. *Hydrobiologia*, 186/187: 81-102.
- Miracle MR, Serra M, Vicente E, Blanco C, 1987. Distribution of *Brachionus* species in Spanish mediterranean wetlands. *Hydrobiologia*, 147: 75-81.
- Miracle MR, Serra M, Oltra R, Vicente E, 1988. Differential distributions of *Brachionus* species in three coastal lagoons. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie*, 23: 2006-2015.
- Modenutti BE, 1998. Planktonic rotifers of Samborombón River Basin (Argentina). *Hydrobiologia*, 387/388: 259-265.
- Moffat BD, Snell TW, 1995. Rapid toxicity assessment using an *in vivo* enzyme test for *Brachionus plicatilis* (Rotifera). *Ecotoxicology and Environmental Safety*, 30: 47-53.
- Montero-Pau J, Gómez A, 2011. Development of genomic resources for the phylogenetic analysis of the *Brachionus plicatilis* species complex (Rotifera: Monogononta). *Hydrobiologia*, 662: 43-50.
- Montero-Pau J, Rasmos-Pérez E, Serra M, Gómez A, 2011. Long-term coexistence of rotifer cryptic species. *PLOS One*, 6: e21530
- Moreno-Garrido I, Lubian LM, Soares AMVM, 1999. *In vitro* populations of rotifer *Brachionus plicatilis* Müller demonstrate inhibition when fed with copper-preaccumulating microalgae. *Ecotoxicology and Environmental Safety*, 44: 220-225.
- Morris RJ, 1974. Lipid composition of surface films and zooplankton from the Eastern Mediterranean. *Marine Pollution Bulletin*, 5: 105-109.
- Moss B, McGowan S, Carvalho L, 1994. Determination of phytoplankton crops by top-down and bottom-up mechanisms in a group of English lakes, the West Midland Meres. *Limnology and Oceanography*, 39: 1020-1029.
- Müller OF, 1786. Animalcula infusoria fluviatilia et marina, quae defexit, systematice descripsit et ad vivum delineari curavit... sistit opus hoc posthumum quod cum tabulis aeneis L. in lucern tradit vidua ejus nobilissima, cura Othonis Fabricii. Haunia; I-LVI + 1-367.
- Navarro N, 1999. Feeding behaviour of the rotifers *Brachionus plicatilis* and *Brachionus rotundiformis* with two types of food: live and freeze-dried microalgae. *Journal of Experimental Marine Biology and Ecology*, 237: 75-87.
- Nixon SW, 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*, 41: 199-219.
- Nogrady T, Wallace RL, Snell TW, 1993. Rotifera, Volume 1: Biology, Ecology and Systematics. In: Dumont HJF, ed. *Guides to the identification of the microinvertebrates of the continental waters of the world, Vol. 4*. SPB Academic Publishing, The Hague, The Netherlands: 142.
- Nowosad P, Kuczyńska-Kippen N, Słodkiewicz-Kowalska A, Majewska AC, Graczyk TK, 2007. The use of rotifers in detecting protozoan parasite infections in recreational lakes. *Aquatic Ecology*, 41: 47-54.
- Ortells R, Snell TW, Gómez A, Serra M, 2000. Patterns of genetic differentiation in resting egg banks of a rotifer species complex in Spain. *Archiv für Hydrobiologie*, 149: 529-551.
- Ortells R, Gómez A, Serra M, 2003. Coexistence of cryptic rotifer species: ecological and genetic characterization of *Brachionus plicatilis*. *Freshwater Biology*, 48: 2194-2202.
- Papakostas S, Triantafyllidis A, Kappas I, Abatzopoulos TJ, 2005. The utility of the 16S gene in investigating cryptic speciation within the *Brachionus plicatilis* species complex. *Marine Biology*, 147: 1129-1139.
- Papakostas S, Dooms S, Christodoulou M, Triantafyllidis A, Kappas I, Dierckens K, Bossier P, Sorgeloos P, Abatzopoulos TJ, 2006a. Identification of cultured *Brachi-*

- onus rotifers based on RFLP and SSCP screening. *Marine Biotechnology*, 8: 547-559.
- Papakostas S, Dooms S, Triantafyllidis A, Deloof D, Kappas I, Dierckens K, De Wolf T, Bossier P, Vadstein O, Kui S, et al., 2006b. Evaluation of DNA methodologies in identifying *Brachionus* species used in European hatcheries. *Aquaculture*, 255: 557-564.
- Papakostas S, Triantafyllidis A, Kappas I, Abatzopoulos TJ, 2009. Clonal composition of *Brachionus plicatilis* s.s. and *B. sp.* 'Austria' hatchery strains based on microsatellite data. *Aquaculture*, 296: 15-20.
- Park GS, Marshall HG, 2000. Estuarine relationships between zooplankton community structure and trophic gradients. *Journal of Plankton Research*, 22: 121-135.
- Patil HS, Meti GM, Hosetti BB, 1993. Biology of multi cell ponds treating municipal wastes. *Internationale Revue der gesamten Hydrobiologie*, 78: 309-317.
- Pejler B, 1995. Relation to habitat in rotifers. *Hydrobiologia*, 313/314: 267-278.
- Persone G, Van de Vel A, Van Steertegem M, De Nayer B, 1989. Predictive value of laboratory tests with aquatic invertebrates: influence of experimental conditions. *Aquatic Toxicology*, 14: 149-166.
- Pintado J, Pérez-Lorenzo M, Luna-González A, Sotelo CG, Prol MJ, Planas M, 2010. Monitoring of the bioencapsulation of a probiotic *Phaeobacter* strain in the rotifer *Brachionus plicatilis* using denaturing gradient gel electrophoresis. *Aquaculture*, 302: 182-194.
- Planas M, Pérez-Lorenzo M, Hjelm M, Gram L, Fiksdal IU, Bergh Ø, Pintado J, 2006. Probiotic effect *in vivo* of *Roseobacter* strain 27-4 against *Vibrio (Listonella) anguillarum* infections in turbot (*Scophthalmus maximus* L.) larvae. *Aquaculture*, 255: 323-333.
- Poole JEP, 1984. A study of the relationship between the mixed liquor fauna and plant performance for a variety of activated sludge sewage treatment works. *Water Research*, 18: 281-287.
- Pourriot R, 1977. Food and feeding habits of Rotifera. *Archiv für Hydrobiologie-Beihefte Ergebnisse der Limnologie*, 8: 243-260.
- Pourriot R, Snell TW, 1983. Resting eggs in rotifers. *Hydrobiologia*, 104: 213-224.
- Price ARG, Nelson-Smith A, 1986. Observations on surface pollution on the Indian Ocean and South China Sea during the Sindbad voyage (1980-81). *Marine Pollution Bulletin*, 17: 60-62.
- Rainuzzo JR, Reitan KI, Olsen Y, 1994a. Effect of short- and long-term lipid enrichment on total lipids, lipid class and fatty acid composition in rotifers. *Aquaculture International*, 2: 19-32.
- Rainuzzo JR, Reitan KI, Jørgensen L, Olsen Y, 1994b. Lipid composition in turbot larvae fed live feed cultured by emulsions of different lipid classes. *Comparative Biochemistry and Physiology*, 107A: 699-710.
- Ricci C, Melone G, 1998. Dwarf males in monogonont rotifers. *Aquatic Ecology*, 32: 361-365.
- Ricci C, Serra M, Snell TW, 2000. Small, beautiful and sexy: what rotifers tell us about ecology and evolution. *Trends in Ecology and Evolution*, 15: 220-221.
- Richardson AJ, 2008. In hot water: zooplankton and climate change. *ICES Journal of Marine Science*, 65: 279-295.
- Rioboo C, Prado R, Herrero C, Cid A, 2007. Population growth study of the rotifer *Brachionus* sp. fed with triazine-exposed microalgae. *Aquatic Toxicology*, 83: 247-253.
- Roche KF, 1995. Growth of the rotifer *Brachionus calyciflorus* Pallas in dairy waste stabilization ponds. *Water Research*, 29: 2255-2260.
- Rodríguez C, Pérez JA, Lorenzo A, Izquierdo MS, Cejas JR, 1994. n-3 HUFA requirement of larval gilthead seabream *Sparus aurata* when using high levels of eicosapentaenoic acid. *Comparative Biochemistry and Physiology*, 107A: 693-698.
- Rodríguez C, Pérez JA, Badía P, Izquierdo MS, Fernández-Palacios H, Lorenzo Hernández A, 1998. The n-3 highly unsaturated fatty acids requirements of gilthead seabream (*Sparus aurata* L.) larvae when using an appropriate DHA/EPA ratio in diet. *Aquaculture*, 169: 9-23.
- Roiha IS, Otterlei E, Samuelsen OB, 2011. Evaluating bioencapsulation of florfenicol in rotifers (*Brachionus plicatilis*). *Aquaculture Research*, 42: 1110-1116.
- Root TL, Price JT, Hall KR, Schneider H, Rosenzweig C, Pounds AJ, 2003. Fingerprints of global warming on wild animals and plants. *Nature*, 421: 57-60.
- Ruttner-Kolisko A, 1974. *Plankton rotifers: biology and taxonomy. Vol. XXVI/1. Die Binnengewässer*, Stuttgart.
- Saksena DN, 1987. Rotifers as indicators of water quality. *Acta Hydrochimica et Hydrobiologica*, 15: 481-485.
- Sarma SSS, Trujillo-Hernández HE, Nandini S, 2003. Population growth of herbivorous rotifers and their predator (*Asplanchna*) on urban wastewaters. *Aquatic Ecology*, 37: 243-250.
- Schmid-Araya JM, 1991. The effect of food concentration on the life histories of *Brachionus plicatilis* (O.F.M.) and *Encentrum linnhei* SCOTT. *Archives of Hydrobiology*, 121: 87-102.
- Schindler DW, 2006. Recent advances in the understanding and management of eutrophication. *Limnology and Oceanography*, 51: 356-363.
- Schröder T, 2005. Diapause in monogonont rotifers. *Hydrobiologia*, 546: 291-306.
- Sen Gupta R, Fondecarr SP, Alagarsamy R, 1993. State of oil pollution in the Northern Arabian Sea after the 1991 Gulf oil spill. *Marine Pollution Bulletin*, 27: 85-91.
- Segers H, 2007. Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa*, 1564: 1-104.

- Serra M, Snell TW, 2009. Sex loss in monogonont rotifers. In: Schön I, Martens K, Van Dijk P, eds. *Lost Sex*. Springer, Berlin: 281-294.
- Serra M, Carmona MJ, Miracle MJ, 1994. Survival analysis of three clones of *Brachionus plicatilis* (Rotifera). *Hydrobiologia*, 277: 97-105.
- Serra M, Galiana A, Gómez A, 1997. Speciation in monogonont rotifers. *Hydrobiologia*, 358: 63-70.
- Serra M, Gómez A, Carmona MJ, 1998. Ecological genetics of *Brachionus* sympatric sibling species. *Hydrobiologia*, 387/388: 373-384.
- Serra M, Snell TW, King CE, 2004. The timing of sex in cyclical parthenogenetic rotifers. In: Moya A, Font E, eds. *Evolution from molecules to ecosystems*. Oxford University Press, New York: 135-146.
- Serrano L, Miracle MR, Serra M, 1986. Differential response of *Brachionus plicatilis* ecotypes to various insecticides. *Journal of Environmental Biology*, 7: 259-275.
- Sharma BK, 1983. The Indian species of the genus *Brachionus* (Eurotatoria: Monogononta: Brachionidae). *Hydrobiologia*, 104: 31-39.
- Skjeremo J, Vadstein O, 1999. Techniques for microbial control in the intensive rearing of marine larvae. *Aquaculture*, 177: 333-343.
- Sládeček V, 1983. Rotifers as indicators of water quality. *Hydrobiologia*, 100: 169-201.
- Sleeter TD, Butler JN, 1982. Petroleum hydrocarbons in zooplankton faecal pellets from the Sargasso Sea. *Marine Pollution Bulletin*, 13: 54-56.
- Smith VH, Joye SB, Howarth RW, 2006. Eutrophication of freshwater and marine ecosystems. *Limnology and Oceanography*, 51: 351-355.
- Snell TW, Boyer E, 1988. Thresholds for mictic female production in the rotifer *Brachionus plicatilis* (Muller). *Journal of Experimental Marine Biology and Ecology*, 124: 73-85.
- Snell TW, Persoone G, 1989. Acute toxicity bioassays using rotifers. I. A test for brackish and marine environments with *Brachionus plicatilis*. *Aquatic Toxicology*, 14: 65-80.
- Snell TW, Carmona MJ, 1995. Comparative toxicant sensitivity of sexual and asexual reproduction in the rotifer *Brachionus calyciflorus*. *Environmental Toxicology and Chemistry*, 14: 415-420.
- Snell TW, Janssen CR, 1995. Rotifers in ecotoxicology: a review. *Hydrobiologia*, 313/314: 231-247.
- Snell TW, Serra M, 2000. Using probability of extinction to evaluate the ecological significance of toxicant effects. *Environmental Toxicology and Chemistry*, 19: 2357-2363.
- Snell TW, Bieberich CJ, Fuerst R, 1983. The effects of green and blue-green algal diets on the reproductive rate of the rotifer *Brachionus plicatilis*. *Aquaculture*, 31: 21-30.
- Snell TW, Moffat BD, Janssen C, Persoone G, 1991. Acute toxicity tests using rotifers. III. Effects of temperature, strain, and exposure time on the sensitivity of *Brachionus plicatilis*. *Environmental Toxicology and Water Quality*, 6: 63-75.
- Snell TW, Kubanek J, Carter W, Payne AB, Kim J, Hicks MK, Stelzer C-P, 2006. A protein signal triggers sexual reproduction in *Brachionus plicatilis* (Rotifera). *Marine Biology*, 149: 763-773.
- Starkweather PL, 1980. Aspects of the feeding behavior and trophic ecology of suspension-feeding rotifers. *Hydrobiologia*, 73: 63-72.
- Starkweather PL, 1987. Rotifera. In: Pandian TJ, Vernberg FJ, eds. *Animal energetics. Vol. 1. Protozoa through Insecta*. Academic Press, Orlando: 159-183.
- Stelzer C-P, 1998. Population growth in planktonic rotifers. Does temperature shift the competitive advantages for different species? *Hydrobiologia*, 387/388: 349-353.
- Stelzer C-P, 2011. The cost of sex and competition between cyclical and obligate parthenogenetic rotifers. *The American Naturalist*, 177: E43-E53.
- Stelzer C-P, Riss S, Stadler P, 2011. Genome size evolution at the speciation level: the cryptic species complex *Brachionus plicatilis* (Rotifera). *BMC Evolutionary Biology*, 11: 90.
- Suatoni E, Vicario S, Rice S, Snell T, Caccone A, 2006. An analysis of species boundaries and biogeographic patterns in a cryptic species complex: The rotifer – *Brachionus plicatilis*. *Molecular Phylogenetics and Evolution*, 41: 86-98.
- Suga K, Mark Welch D, Tanaka Y, Sakakura Y, Hagiwara A, 2007. Analysis of expressed sequence tags of the cyclically parthenogenetic rotifer *Brachionus plicatilis*. *PLOS One*, 2: e671.
- Suga K, Mark Welch DB, Tanaka Y, Sakakura Y, Hagiwara A, 2008. Two circular chromosomes of unequal copy number make up the mitochondrial genome of the rotifer *Brachionus plicatilis*. *Molecular Biology and Evolution*, 25: 1129-1137.
- Suzer C, Çoban D, Kamaci OH, Saka Ş, Firat K, Otgucuoğlu Ö, Küçüksarı H, 2008. *Lactobacillus* spp. bacteria as probiotics in gilthead sea bream (*Sparus aurata*, L.) larvae: effects on growth performance and digestive enzyme activities. *Aquaculture*, 280: 140-145.
- Tamplin ML, Gauzens AL, Huq A, Sack DA, Colwell RR, 1990. Attachment of *Vibrio cholerae* serogroup 01 to zooplankton and phytoplankton of Bangladesh waters. *Applied and Environmental Microbiology*, 56: 1977-1980.
- Thassitou PK, Arvanitoyannis IS, 2001. Bioremediation: a novel approach to food waste management. *Trends in Food Science & Technology*, 12: 185-196.
- Timms BV, 1993. Saline lakes of the Paroo, inland New South Wales, Australia. *Hydrobiologia*, 267: 269-289.
- Tinh NTN, Phuoc NN, Dierckens K, Sorgeloos P, Bossier P, 2006. Gnotobiotically grown rotifer *Brachionus plicatilis* sensu stricto as a tool for evaluation of microbial functions and nutritional value of different food types. *Aquaculture*, 253: 421-432.

- Tinh NTN, Linh ND, Wood TK, Dierckens K, Sorgeloos P, Bossier P, 2007. Interference with the quorum sensing systems in a *Vibrio harveyi* strain alters the growth rate of gnotobiotically cultured rotifer *Brachionus plicatilis*. *Journal of Applied Microbiology*, 103: 194-203.
- Trout JM, Walsh EJ, Fayer R, 2002. Rotifers ingest *Giardia* cysts. *Journal of Parasitology*, 88: 1038-1040.
- Turner PN, 1993. Distribution of rotifers in a Floridian salt-water beach, with a note on rotifer dispersal. *Hydrobiologia*, 255/256: 435-439.
- Turner JT, Tester PA, 1992. Zooplankton feeding ecology: bacterivory by metazoan microzooplankton. *Journal of Experimental Marine Biology and Ecology*, 160: 149-167.
- Turner JT, Tester PA, 1997. Toxic marine phytoplankton, zooplankton grazers, and pelagic food webs. *Limnology and Oceanography*, 42: 1203-1214.
- Turton CL, McAndrews JH, 2006. Rotifer loricas in second millennium sediment of Crawford Lake, Ontario, Canada. *Reviews of Palaeobotany and Palynology*, 141: 1-6.
- Uhlmann D, 1980. Limnology and performance of waste treatment lagoons. *Hydrobiologia*, 72: 21-30.
- Vasileiadou K, Papakostas S, Triantafyllidis A, Kappas I, Abatzopoulos TJ, 2009. A multiplex PCR method for rapid identification of *Brachionus* rotifers. *Marine Biotechnology*, 11: 53-61.
- Verpraet R, Chair M, Leger P, Nelis H, Sorgeloos P, De Leenheer A, 1992. Live-food mediated drug delivery as a tool for disease treatment in larviculture. The enrichment of therapeutics in rotifers and *Artemia* nauplii. *Aquacultural Engineering*, 11: 133-139.
- Walker KF, 1981. A synopsis of ecological information on the saline lake rotifer *Brachionus plicatilis* Müller 1786. *Hydrobiologia*, 81: 159-167.
- Wallace RL, 2002. Rotifers: exquisite metazoans. *Integrative and Comparative Biology*, 42: 660-667.
- Wallace RL, Snell TW, 1991. Rotifera. In: Thorpe JH, Covich AP, eds. *Ecology and classification of North American freshwater invertebrates*. Academic Press Inc, New York: 187-248.
- Wallace RL, Smith HA, 2009. Rotifera. In: Likens GE, ed. *Encyclopedia of inland waters*. Oxford, Elsevier: 689-703.
- Wallace RL, Snell TW, Ricci C, Nogrady T, 2006. *Rotifera I: Biology, Ecology and Systematics*. Backhuys Publishers, Leiden, The Netherlands.
- Walsh EJ, Schröder T, Wallace RL, Ríos-Arana JV, Rico-Martínez R, 2008. Rotifers from selected inland saline waters in the Chihuahuan Desert of Mexico. *Saline Systems*, 4: 7-18.
- Walther G-R, Post E, Convey P, Menzel A, Parmesan C, Beebee TJC, Fromentin J-M, Hoegh-Guldberg O, Bairlein F, 2002. Ecological responses to recent climate change. *Nature*, 416: 389-395.
- Wang L, Yan T, Yu R, Zhou M, 2005. Experimental study on the impact of dinoflagellate *Alexandrium* species on populations of the rotifer *Brachionus plicatilis*. *Harmful Algae*, 4: 371-382.
- West SA, Lively CM, Read AF, 1999. A pluralist approach to sex and recombination. *Journal of Evolutionary Biology*, 12: 1003-1012.
- Winder M, Schindler DE, 2004. Climate change uncouples trophic interactions in an aquatic system. *Ecology*, 85: 2100-2106.
- Wolfe MF, Schlosser JA, Schwartz GJB, Singaram S, Mielbrecht EE, Tjeerdema RS, Sowby ML, 1998. Influence of dispersants on the bioavailability and trophic transfer of petroleum hydrocarbons to primary levels of a marine food chain. *Aquatic Toxicology*, 42: 211-227.
- Wrona FJ, Prowse TD, Reist JD, Hobbie JE, Lévesque LMJ, Vincent WF, 2006. Climate change effects on aquatic biota, ecosystem structure and function. *Ambio*, 35: 359-369.
- Wyngaard GA, Rasch EM, Manning NM, Gasser K, Domangue R, 2005. The relationship between genome size, development rate, and body size in copepods. *Hydrobiologia*, 532: 123-137.
- Xie Z, Xiao H, Tang X, Lu K, Cai H, 2008. Interactions between red tide microalgae and herbivorous zooplankton: effects of two bloom-forming species on the rotifer *Brachionus plicatilis* (O.F. Müller). *Hydrobiologia*, 600: 237-245.
- Yasuno M, Asaka A, Kono Y, 1993. Effects of pyraclofos (an organophosphorous insecticide) on nutrient enriched ecosystems. *Chemosphere*, 27: 1813-1824.
- Yoshida K, Ishii H, Ishihara Y, Saito H, Okada Y, 2009. Bioremediation potential of formaldehyde by the marine microalga *Nannochloropsis oculata* ST-3 strain. *Applied Biochemistry and Biotechnology*, 157: 321-328.
- Yoshinaga T, Hagiwara A, Tsukamoto K, 2000. Effect of periodical starvation on the life history of *Brachionus plicatilis* O.F. Müller (Rotifera): a possible strategy for population stability. *Journal of Experimental Marine Biology and Ecology*, 253: 253-260.
- Yoshinaga T, Kaneko G, Kinoshita S, Tsukamoto K, Watabe S, 2003. The molecular mechanisms of life history alterations in a rotifer: a novel approach in population dynamics. *Comparative Biochemistry and Physiology B*, 136: 715-722.
- Zakaria HY, Hussien Ahmed M, Flower R, 2007. Environmental assessment of spatial distribution of zooplankton community in Lake Manzalah, Egypt. *Acta Adriatica*, 48: 161-172.
- Zhao Q, Wang B, 1996. Evaluation on a pilot-scale attached-growth pond system treating domestic wastewater. *Water Research*, 30: 242-245.
- Zou Y, Yamasaki Y, Matsuyama Y, Yamaguchi K, Honjo T, Oda T, 2010. Possible involvement of hemolytic activity in the contact-dependent lethal effects of the dinoflagellate *Karenia mikimotoi* on the rotifer *Brachionus plicatilis*. *Harmful Algae*, 9: 367-373.