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***Corbicula fluminea* invasion in Lake Maggiore:
a three years ecological study**

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The number of reports of non-indigenous species (NIS) of the aquatic environments are increasing rapidly worldwide. The rate of invasions of freshwater environments is rising in many countries, mainly caused by an increase in human activities. The growing interest in invasive species is due to their negative impact on biodiversity and ecosystem functioning. Some species are recognized among the most dangerous invasive species of freshwater ecosystems (eg *C. fluminea*, *D. polymorpha* and *A. woodiana*).

This study aims to evaluate the *C. fluminea* invasion in Lake Maggiore, inspecting different characteristics of populations settled in southern littoral part. Field and semi-natural condition observations are carried out to evaluate growth and reproductive rate of *Corbicula* in the environment recently colonized. Lab experiments were performed to measure filtration rate. To evaluate possible competitive interactions between alien and native species, dietary sources and trophic niche overlap were determined through measurements of the stable isotope compositions of C ($\delta^{13}\text{C}$) and N ($\delta^{15}\text{N}$) respectively.

Lake Maggiore offers a favorable environment for the establishment of the Asian clam *Corbicula fluminea*. The picture that emerges from the study of *C. fluminea* populations is quite complex, with differences among the five sampling sites. Larger size class organisms are well represented in Angera and Arona (southern sites), while are (still) almost absent in Feriolo and Monvalle (northern sites).

The density and biomass of *C. fluminea* observed are rather high, with peaks reaching up to 3000 ind.m⁻² and 2500 g m⁻².

The populations size structure provides clues to understand the process of invasion and its phases. The populations size structure in the different sites presents wide differences, indicative of different demographic dynamics. The timing of colonization of Lake Maggiore seems to follow this order: Monvalle <Feriolo <Brebbia <Angera <Arona. It is also possible to assume that the colonization of this environment began in 2007, for expansion of the population already established in its effluent, the river Ticino.

The reproductive period of *C. fluminea* in Lake Maggiore showed a single reproductive peak, from late Spring to Autumn. The recruitment of new organisms is scarce at the site of Arona, while the opposite situation is observed in Feriolo.

The number of larvae produced by each adult is highly variable, with values ranging from a few hundred to 16000 larvae per individual.

Generally in the sites of Angera, Brebbia, Feriolo and Monvalle mortality is rather low, while at Arona is considerably higher. At Angera was observed a phenomenon of mass mortality in Autumn 2012.

C. fluminea has a high growth rate and in this environment there are no factors able to limit the growth and spread of this species. The growth rates are affected by seasonal variations in temperature: in Summer and Autumn organisms show higher growth rate values than in the other seasons (almost 2 times higher). The growth rate is higher for small size organisms, as expected comparing young and adults organisms of the same species.

In spite of similar functional roles, Unionids and *C. fluminea* are likely to differ in filtering efficiency and trophic niche. Our preliminary results suggest that *Corbicula* has a noticeably higher filtration capacity than the most abundant Unionid species (*U. elongatulus*) and is able to shift its diet along the year. The results of stable isotope analysis highlight a niche overlap between *C. fluminea* and native species of bivalves, indicating that *C. fluminea* has lower trophic fidelity than native Unionids. Feeding plasticity of *C. fluminea* is likely of major importance for competing successfully indigenous bivalves in Lake Maggiore, thus promoting the overdomination of the alien species in the area. Indeed, *Corbicula* seems to stand already as a keystone littoral species in Lake Maggiore, likely being a strong competitor for space and food to native mussels.

However, besides the competition with bivalve invasive species the decline observed for the native species of Lake Maggiore (*U. elongatulus* abundance decreases about 90 % from 2004 and 2012) is likely due to several factors. Severe drought is responsible for periodical mass mortalities which affect both native Unionids and invasive clams, but these latter can recover and recolonize the environment more rapidly than native species.

The specimens that moving from one environment to another can bring along their parasites, which can find the conditions necessary to establish a new population. In the course of this study two new species of parasites of Unionids (cercariae of Bucephalidae and *Rhodeus amarus*) have been found. The infection of the gonads due to cercariae leads to the castration of bivalves and can be fatal, while the presence of the larvae of *R. amarus* in the gills reduces the rate of respiration and filtration.

The presence of *D. polymorpha* is recognized in the lake since the 1990's and is often found anchored to the shell of other bivalve species. High infestation of *Dreissena* can cause the death by asphyxia.

The combination of all these factors provides sufficient explanation for the observed decline of native species, however, the invasion of *C. fluminea* appears to be a worrying phenomenon. After 3-4 years from the entry into the lake this clam had already colonized a third of the shoreline. To date, this bivalve is found in all suitable habitats distributed along the entire perimeter of the lake.

It is possible to conclude that eradication is an expensive solution and the methodologies used often have negative impacts on native species. Moreover, it is not possible to eradicate a species from the environment, in addition the high growth rate and the plasticity of the alien species recorded in Lake Maggiore favour them respect native species. Mass mortality events can promote the replacement of the native by alien species.

1 Introduction

Animal and plant species adopt different methods to spread naturally in the environment and occupy different habitats. However, globalization has led to the development of trade and travel, facilitating the movement of goods and people on the planet surface, bringing as a side effect the spread of non-native species around the world (Keller *et al.*, 2011). The invasions are a significant component of global environmental change (Marchetti *et al.*, 2004) producing considerable ecological impacts, genetic, economic and social. These species can act as vectors of introduction of disease, can change ecosystems, reduce biodiversity and cause huge economic damage (Sousa *et al.*, 2008a; Moyle & Light, 1996). The history of the human being is characterized by its need to explore, communicate and trade. The migration of people, in ancient times, was sometimes responsible for the translocation of biological species in new areas. The frequency of introductions has increased significantly over the past four centuries, in association with the people migrations from Europe to other continents. It is expected that, with the evolution of technologies applied to transport, distances seem to shrink, reducing journey times and making services more accessible to a growing number of users. In addition, global climate change might increase the susceptibility of environments for the invasion, the frequency of invasions is likely to increase (de Moor, 1993).

A new species can be introduced into a new environment deliberately or accidentally. Not all species that are released find conditions that ensure their survival and reproduction. According to Williamson & Fitter (1996) 10% of the imported species escape the control conditions and only 10% of these are able to establish a population and become naturalized. 10% of these naturalized species find suitable conditions as to expand, invading the new environment.

The terms alien, non-native, non-indigenous and exotic are used interchangeably to describe the species that are established in areas temporally and spatially distinct from their habitat original (Occhipinti-Ambrogi & Galil, 2004; Colautti & MacIsaac, 2004; Davis & Thompson, 2000). The International Union for Conservation of Nature and Natural Resources (IUNC 2000) defines invasive species the non-native species able to settle in the new natural or semi-natural habitats and become an agent of disturbance of native species and original biodiversity.

Anthropogenic influence can act directly, with a facilitation of the spread of the species, or indirectly, with the modification of ecosystems or the alteration of the structure of indigenous communities.

The introductions can be voluntary or non voluntary. For example, with the abandonment of live bait, release into the wild to domestic animals or cultivating the seeds collected during a trip, just to name a few of voluntary introductions. The type of unintentional introductions can follow different routes: organisms can escape from farming or agriculture; larvae and spores can be transported over long distances hidden within the goods, on the surface of or inside vehicles.

This phenomenon affects all environments, both terrestrial and aquatic, marine and freshwater. The rate of invasions of freshwater environments is increasing in many countries, mainly due to an increase in transport (Oscoz *et al.*, 2010).

The number of reports of non-indigenous species (NIS) of the aquatic environments are increasing rapidly worldwide (DAISIE, 2008) emphasizing the public and scientific awareness of the real threats that invasive species cause on biodiversity and ecosystem functioning (Sousa *et al.*, 2011).

The interest related to the phenomenon of the invasion ecology has grown tremendously over the last 50 years since the book of Elton " The ecology of invasions by animals and plants " (Elton 1958) was published (reviewed in Richardson & Pysek, 2008).

This is becoming an important multidisciplinary subfield of ecology with growing links to many other disciplines, with the continuous improvement of the methods and techniques that relate to the introduction and establishment of organisms into new environments, their interactions with living organisms, and the concern of the costs and benefits of their presence and abundance with reference to systems of human values (Richardson & van Wilgen, 2004; Richardson & Pysek, 2008).

Several aspects of the dynamics of invasions need of further investigation (Williamson, 1999; Hayes & Barry, 2008). In particular, the dynamics of the new entry has been rarely documented. Invasive species are difficult to detect at early stage of colonization; usually attract attention only after the population has been established and when the impact is already quite serious.

Recent studies confirm that the success of insects, zooplankton, fishes, birds and mammals is related to propagule pressure (Lockwood *et al.*, 2005), which considers the size of propagules (number of introduced organisms in the new environment) and the number of propagules (the number of events of introduction) (Kolar & Lodge, 2001; Cassey *et al.*, 2004). Understanding the propagule pressure is a relatively new frontier in the ecology of the invasion (Richardson, 2004), and provides a key element in explaining why some populations persist, while others are not able to establish (Kolar & Lodge 2001, Lockwood *et al.*, 2005).

Generally, just settled population consists of a few individuals and can easily go towards extinction. The early stages of the process of invasion are particularly critical. Three barriers to be overcome have been recognized as main steps for establishment of the population in the new environment. The first consists in finding an appropriate niche, allowing the survival and reproduction of the propagules. Secondly, the reproduction rate must be high enough to offset the mortality (second barrier). Finally, the third barrier is over when the population has grown sufficiently in number to avoid extinction caused by stochastic events due to demographics, environment or to the genetic pool of the population (Sol, 2007).

The process of invasion may be subdivided into distinct phases: new introduced species afford an acclimatization phase during which it is not observed massive growth (*lag* phase or latency), which corresponds to overcoming the first barrier. Following starts a phase of

exponential growth and number of individuals greatly increases (*log* phase), allowing the overcoming of the second and third barrier. After a few generations (the number is determined by many different factors as the number of new born per generation and the environmental characteristics) tends to stabilize the population numbers on stable values (*plateau* phase) and then, in some cases, decrease arriving to a phase of decline which can lead to extinction (Nentwig & Josefsson, 2010).

In literature, are reported several hypothetical characteristics which identify a "successful invader ". Pre-adaptation to the new environment gives some advantages to the species. For example, the generalist species are characterized by having a broad trophic niche and a low selectivity towards the food source. Also the behavioral and social flexibility provides the ability to better adapt to a new environment. In the early stages of settlement, species characterized by longevity and with a high rate of growth are more successful, quickly reaching an abundant population. In addition, the achievement of early maturity, low selectivity and sexual hermaphroditism are characteristics that increase the success of species introduced into a new environment (Sol, 2007).

The introduction of alien species in freshwater environments can occur in several ways: for example, with canalization (Oscoz *et al.*, 2010), through ship ballast water, released from aquaria or garden ponds and with the release of baits (Strayer, 2010; Lodge *et al.*, 1998).

Considering the freshwater, some bivalve species are recognized among the most dangerous invasive species:

- *Corbicula fluminea* (Müller, 1774) is a bivalve native to South-East Asia. In the '20s is found in North America and in about 50 years colonizes all the lakes and rivers south of 40° parallel (Britton & Morton, 1982). Among the 60's and '70s *C. fluminea* also invades the South America: northern Argentina, Uruguay, Paraguay and southern Brazil (Cataldo & Boltovskoy, 1998). In the '70s *Corbicula* is found in Europe in the area Caspian-Caucasian. In the two decades following the bivalve also colonizes France, Portugal, the Netherlands, Germany, Spain and Belgium (Ciutti *et al.*, 2007; Araujo *et al.*, 1993). In Italy *C. fluminea* is detected in the Po River around 1995 (Bedulli *et al.*, 1995; Fabbri & Landi, 1999; Malavasi *et al.*, 1999). This species is present in Lake Garda, in the southern area (Nardi & Braccia, 2004) and colonizes the Lago Maggiore from 2007 (Kamburska *et al.*, 2013a).
- *Anodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) is native to East Asia (from southeastern Russia to Malaysia). Over the past few years, has been detected throughout Europe (Kiss, 1990; Paunović *et al.*, 2006; Popa *et al.*, 2007; Munjiu & Shubernetski, 2008; Pou-Rovira *et al.*, 2009; Lajtner & Crnčan, 2011; Sárkány-Kiss *et al.*, 2000). The first detection in Italy (Manganelli *et al.*, 1998) is followed by a growing number of records. To date, the actual distribution seems to cover the entire peninsula from north to south (Manganelli *et al.*, 1998; Bodon *et al.*, 2005, Solustri & Nardi, 2006; Cianfanelli *et al.*, 2007; Albano, 2006; De Vico *et al.*,

2007). The *A. woodiana* has colonized the great Italian lakes, such as Lake Garda (Cappelletti *et al.*, 2009) and Lake Maggiore (Kamburska *et al.*, 2013b).

- *Dreissena polymorpha* (Pallas, 1771) is a bivalve native to the Caspian-Caucasian region that has a global distribution. Colonizes the most diverse environments, both lotic and lentic, and causes extensive damage since, reaching very high densities, can obstruct water intakes and canals, or anchor to the hulls of boats, with consequent expensive works of maintenance (Schalekamp 1971, Greenshields & Ridley, 1957).

1.1 Aim of the study

This study was performed to evaluate the dynamics of invasion of *C. fluminea* in Lake Maggiore, observing the process from its initial phase. Five sampling sites were defined in the southern littoral area. Density, biomass and population size structure were analysed seasonally. Besides assessing *C. fluminea* current spatial distribution, the age/size distribution and abundance of the populations established in different sites along the lake littoral were assessed to trace the colonization history of this species in Lake Maggiore.

Growth rate and reproduction data in field were compared with those measured in semi-natural conditions. Finally, an experimental part was carried out to analyse the filtration rate and stable isotope composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of alien and native species of Lake Maggiore and to evaluate possible competitive interactions between these two groups of molluscs.

2. Materials and Methods

2.1 *Alien and Native molluscs species of the lake*

2.1.1 *Morphology and Anatomy*

The bivalves consist exclusively of aquatic molluscs; most of species are marine. The name is derived from shell, composed of two parts named valves. The opening and closing of valves is mediated by the adductor muscles. Inside, immediately below the shell is present the mantle, deputy to the formation of new shell. In the central part there is the visceral sac and the foot. The head is not present and the nervous system is poorly developed. The two pairs of gills (inner and outer demibranchs) are located between the mantle and the visceral sac. Gills have respiratory and feeding function, they are used from bivalves as natural filter. Moreover gills have reproductive functions, inside the inner or the outer demibranch (depending on species) occurs the larval development (Tachet *et al.*, 2000; Campaioli *et al.*, 1994).

2.1.2 *Biology*

Bivalves are generally sexes separate with no evidence of sexual dimorphism (Unionoidea (Figures from 5 to 8) and Dreissenacea (Figure 4)) with the exception of Sphaeriidae that are hermaphrodites.

Fertilization is water mediated, the male gametes are released to reach the female ones. Fertilization can take place directly in the water or in the mantle cavity (Campaioli *et al.*, 1994).

Regarding *C. fluminea* larval development occurs within the inner demibranchs (Figure 1), the *larvae* are released into the environment in the form of *pediveliger* (250 μm), they are already formed and have the usual D-shaped conformation (Sousa *et al.*, 2008a; Britton & Morton, 1982). *C. fluminea* (Figure 3) is an hermaphroditic species that can self-fertilize, and it is also seem to reproduce through androgenesis (Pigneur *et al.*, 2012; Hedtke *et al.*, 2008; Ishibashi *et al.*, 2003).

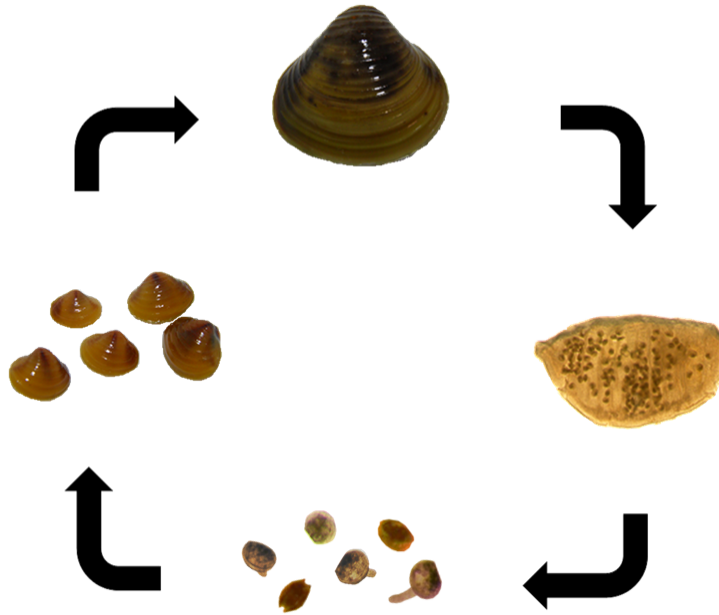


Figure 1: Reproductive cycle of *C. fluminea*
(modified from Sousa *et al.*, 2008a)

The Unionoidea produces larvae within the pair of external gills and release a larval stage, called glochidia, which needs a host to complete its development (Figure 2). Generally glochidia attaches to the gills or fins of fish, the choice of the host is related to mussel species, although some molluscs species are less selective than others. The glochidia encysts in the tissues of fish and, after a period of time (variable from species to species), the development is complete. The young molluscs are detached from the fish tissues and fall in the sediment, where they begin to conduct benthic life (Tachet *et al.*, 2000; Campaioli *et al.*, 1994).

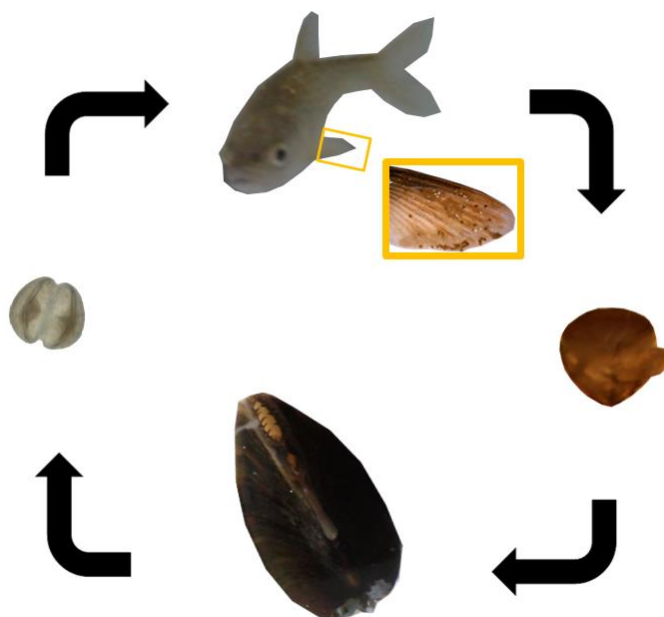


Figure 2: Reproductive cycle of Unionoidea

2.1.3 Ecology

The Unionoidea are generally quite tolerant to environmental conditions, in fact are able to resist to low oxygen conditions or high load of pollutants (Campaioli *et al.*, 1994). Bivalves are filter feeders and feed on planktonic organisms, but also of bacteria and organic detritus. The Unionoidea, as for other bivalves (e.g. genus *Corbicula*, superfamily Corbiculoidea), are able to feed with the organic substance contained in the sediment with a mechanism called pedal feeding (Werner & Rothhaupt, 2007).

The adults are sessile, therefore the dispersion is mostly linked to juvenile stages that are the ones who can easily travel for long distances (e.g. transported by ballast water).

The larvae of *Corbicula* released into the environment are able to anchor to the sediment or vegetation by a single mucilaginous byssal threads (Britton & Morton, 1982). For Unionoidea the dispersion of glochidia is mediated by the fishes that are hosting them.

Bivalves can be preyed on by several species of crustaceans, aquatic birds, like swans and coots, and also by fishes (Tachet *et al.*, 2000).

2.1.4 Invasion

C. fluminea is recognized as one of the most "efficient" invaders freshwater, among the 100 worst invasive alien species (DAISIE 2008). *Corbicula* can survive in a wide range of environmental conditions due to its high ecological and physiological plasticity (Byrne and McMahon, 1983; Johnson & McMahon, 1998; McMahon, 2000; McMahon & Bogan, 2001, Sousa *et al.*, 2007, 2008a, b). Features such as a short life cycle (a mean of 1 to 5 years, and in any case does not seem to exceed 7 years (Hall, 1984)), high fecundity, rapid growth, dispersal ability (especially typical of larvae) and opportunistic behavior are typical r- strategy, and of this species (Sousa *et al.*, 2008a).

In literature there are many articles that illustrate the impact of *Corbicula* on specific ecosystem properties (reviewed by Sousa *et al.*, 2008a), such as the alteration of the nutrient cycle (Beaver *et al.*, 1991), phytoplankton consumption (Cohen *et al.*, 1984), competition for resources and space with native macroinvertebrates and substrate modification (Werner & Rothhaupt, 2007, 2008; Karatayev *et al.*, 2007). Also human activities are subject to a strong impact, especially due to biofouling (Mattice 1979, McMahon 1983; Darrigran 2002). The larvae can disperse naturally due to water currents (Figuerola & Green 2002). The production of byssal threads is implicated in the spread of juvenile stages of *C. fluminea*, which are anchored to floating debris, but also to feathers and legs of aquatic birds (McMahon, 1999).

The accidental introduction of fish infested by glochidia (parasitic larval form) seems to be a reasonable vehicle for rapid expansion of *A. woodiana* in European countries (Spyra *et al.*, 2012) and Italy (Gherardi *et al.*, 2008; Cianfanelli *et al.*, 2007). *A. woodiana* is a host-generalist, able to parasitize different species of fish, native or invasive; this attitude increased opportunities to reach and establish in new environments (Douda *et al.*, 2012).

The main routes of spread of *D. polymorpha* are recognized in the inland, especially after the opening of new waterways between Eastern and Central Europe in the early 1800s. Another vehicle of introduction is recognized in the transfer of animals (including clayfish) for storage on aquaculture. The introduction of mussels in the lakes Europeans and Americans is conveyed by boat hulls where mussel anchor itself; the increase of the recreational ships has been observed during the post-war period (Birnbaum, 2011) and facilitate this process.

2.1.5 Taxonomy

Corbicula fluminea



Figure 3: *Corbicula fluminea*

Phylum: Mollusca (Linnaeus, 1758)

Class: Bivalva (Linnaeus, 1758)

Subclass: Heterodonta (Neumayr, 1884)

Order: Veneroida (H. & A. Adams, 1856)

Superfamily: Corbiculoidea (Gray, 1847)

Family: Corbiculidae (Gray, 1847)

Genus: *Corbicula* (von Mühlfeld, 1811)

Species: *fluminea* (Müller, 1774)

Dreissena polymorpha



Figure 4: *Dreissena polymorpha*

Phylum: Mollusca (Linnaeus, 1758)

Class: Bivalva (Linnaeus, 1758)

Subclass: Heterodonta (Neumayr, 1884)

Order: Veneroida (H. & A. Adams, 1856)

Superfamily: Dreissenoidea (Gray, 1840)

Family: Dreissenidae (Gray, 1840)

Genus: *Dreissena* (Beneden, 1835)

Species: *polymorpha* (Pallas, 1771)

Anodonta woodiana



Figure 5: *Anodonta woodiana*

Phylum: Mollusca (Linnaeus, 1758)

Class: Bivalva (Linnaeus, 1758)

Subclass: Palaeoheterodonta (Newell, 1965)

Order: Unionoida (Stoliczka, 1871)

Superfamily: Unionoidea (Rafinesque, 1820)

Family: Unionidae (Rafinesque, 1820)

Genus: *Anodonta* (Lamarck, 1799)

Species: *woodiana* (Lea, 1834)

Anodonta anatina



Figure 6: *Anodonta anatina*

Phylum: Mollusca (Linnaeus, 1758)

Class: Bivalva (Linnaeus, 1758)

Subclass: Palaeoheterodonta (Newell, 1965)

Order: Unionoida (Stoliczka, 1871)

Superfamily: Unionoidea (Rafinesque, 1820)

Family: Unionidae (Rafinesque, 1820)

Genus: *Anodonta* (Lamarck, 1799)

Species: *anatina* (Linnaeus, 1758)

Anodonta cygnea



Figure 7: *Anodonta cygnea*

Phylum: Mollusca (Linnaeus, 1758)

Class: Bivalva (Linnaeus, 1758)

Subclass: Palaeoheterodonta (Newell, 1965)

Order: Unionoida (Stoliczka, 1871)

Superfamily: Unionoidea (Rafinesque, 1820)

Family: Unionidae (Rafinesque, 1820)

Genus: *Anodonta* (Lamarck, 1799)

Species: *cygnea* (Linnaeus, 1758)

Unio elongatulus



Figure 8: *Unio elongatulus*

Phylum: Mollusca (Linnaeus, 1758)

Class: Bivalva (Linnaeus, 1758)

Subclass: Palaeoheterodonta (Newell, 1965)

Order: Unionoida (Stoliczka, 1871)

Superfamily: Unionoidea (Rafinesque, 1820)

Family: Unionidae (Rafinesque, 1820)

Genus: *Unio* (Philipsson, 1788)

Species: *elongatulus* (Pfeiffer, 1825)

2.2 *Study area*

2.2.1 *Lake Maggiore*

Lake Maggiore (latitude N 45°50'19"; longitude E 8°37'17") is a tectonic-glacially formed lake in the southern side of Lepontine Alps (Figure 9). This lake presents the biggest basin catchment (6.599 km²) among Italian lakes, equally divided between Italy and Switzerland. Major tributaries are River Ticino (inflow), River Maggia and River Toce, others eleven minor tributaries bring water to the lake; River Ticino is the only outflow, located in the southern part of the lake.

Lake Maggiore is the second Italian lake considering surface (212.5 km²) and maximum depth (372 m). Before the 1960's this lake was classified as oligotrophic. Sign of changes were detected with increase of nutrients concentration and appearance of *Oscillatoria rubescens* (Ravera & Vollenweider, 1968); in the middle of 1970's trophic level was considered as meso-eutrophic. As a results of actions to reduce the supply of nutrients; the lake is currently considered as oligo-mesotrophic (Calderoli *et al.*, 1997; Salmaso & Mosello, 2010).

Today Lake Maggiore is known for his beauty and wonderful landscape, is popular for tourists from all over Europe: resident population of the lake pass from 670'000 to 12 million daily presence during Summer, concentrated on shoreline area (De Bernardi *et al.*,

1999). Shoreline covers 170 km and presents a wide range of different habitats. Steep and rocky banks mainly characterize the upper (northern) part of the lake; except for the area of the Magadino floodplain (Canton Ticino, Switzerland). In contrast, the southern is characterised by gentle slopes with a soft to sandy-gravelly bottom. The largest proportion of inhabitants and industries is distributed in the southern part of the catchment area.

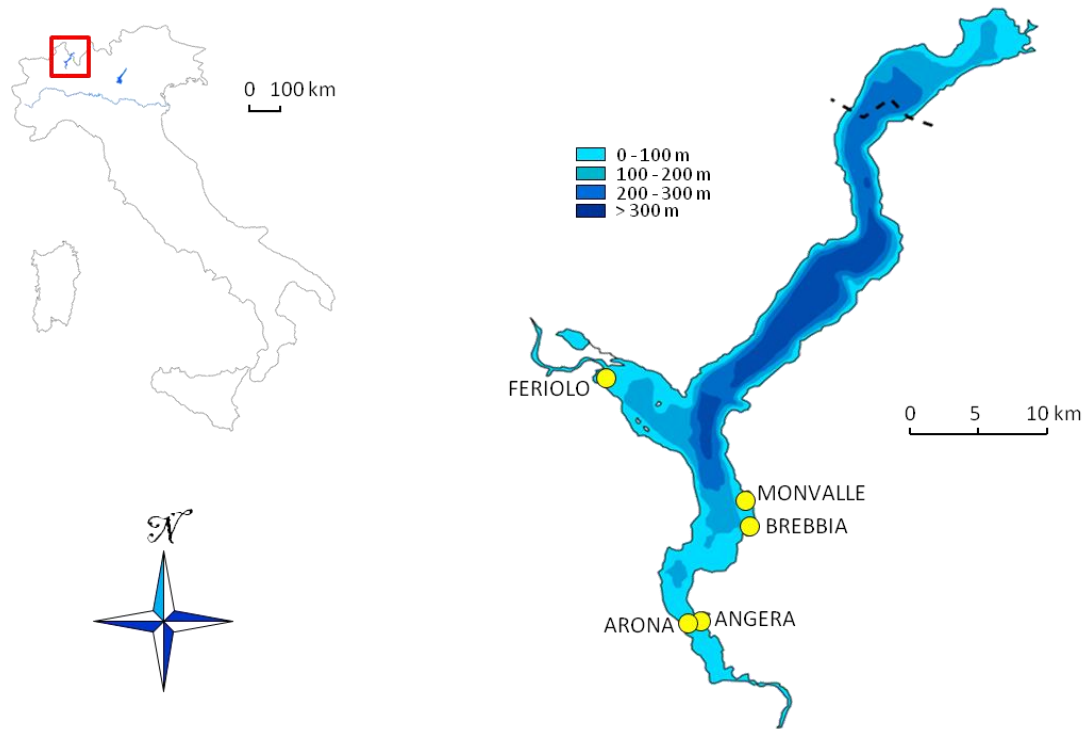


Figure 9: Freshwater bivalves sampling sites of Lake Maggiore

Table 1: Sampling sites and their coordinates as decimal degrees

Site	Coordinates	
Angera	45° 46' 14"	8° 34' 34"
Arona	45° 44' 58"	8° 34' 01"
Brebbia	45° 50' 04"	8° 37' 21"
Feriolo	45° 55' 55"	8° 28' 59"
Monvalle	45° 51' 02"	8° 37' 07"

Lake Maggiore is already colonized by different alien species, for example: *Rutilus rutilus* (Volta & Jepsen, 2008), *Eudiaptomus gracilis* (Riccardi & Rossetti, 2007), *Polyphemus pediculus* (Visconti *et al.*, 2011), *Orconectes limosus* (Casale & Brambilla, 2010). For what concerns molluscs, the zebra mussel *Dreissena polymorpha* was accidentally introduced likely in the 1990's, while the Asian clam *Corbicula fluminea* and the Chinese pond mussel *Anodonta woodiana* were recorded for the first time in 2010 (Kamburska *et al.*, 2013a; Kamburska *et al.*, 2013b).

Dominant native molluscs species present in lake Maggiore, belong to the family Unionidae, with one species of genus *Unio* (*U. elongatulus*) that is the most abundant and two species of *Anodonta* (*A. cygnea* and *A. anatina*) that are more rare (Riccardi, personal data).

2.2.2 Sampling sites

In September 2010, 30 locations around the lake perimeter were monitored to trace alien species diffusion along the shoreline (Kamburska *et al.*, 2013a). Shallow areas were visually inspected, while scuba dive surveys were undertaken in the deeper areas up to 15 meters depth. A large variety of littoral habitats was investigated and for the first quantitative sampling 4 sampling sites were chosen (Figure 9, Table 1). These areas are located in the southern part of the lake, two on Piedmont shore (Arona and Feriolo, western side) and the other two on Lombardy shore (Angera and Brebbia, eastern site).

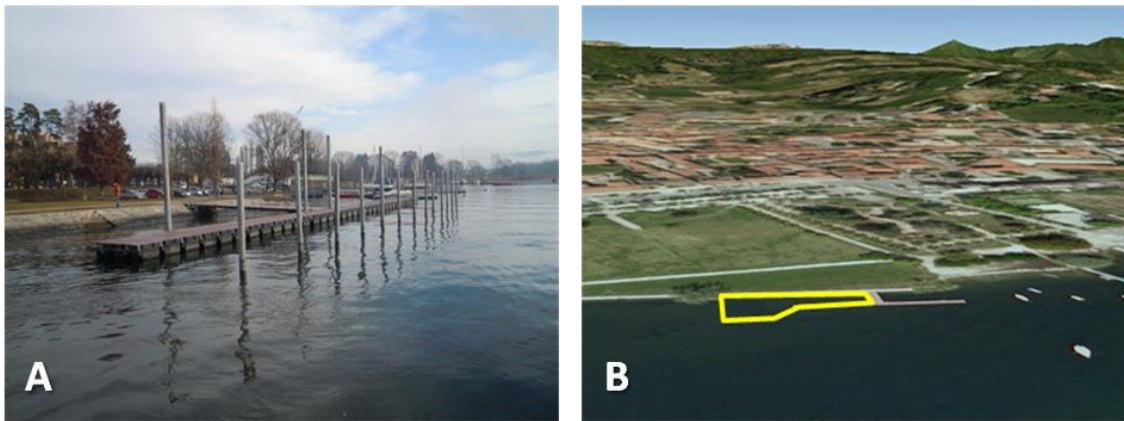


Figure 10: Angera

A - Picture of floating pier in front of Angera village

B - Google maps image with sampling area highlighted in yellow

Angera (Figure 10): site subjected to a high degree of anthropization, where heavy recreational boat traffic is present. Shoreline is defined by a concrete wall which is connected to a floating pier. Nearby there are a boating and the village centre. Shore becomes steep below the pier and sediments are composed by stones covered with abundant deposition of coarser sediment, poor in silt.

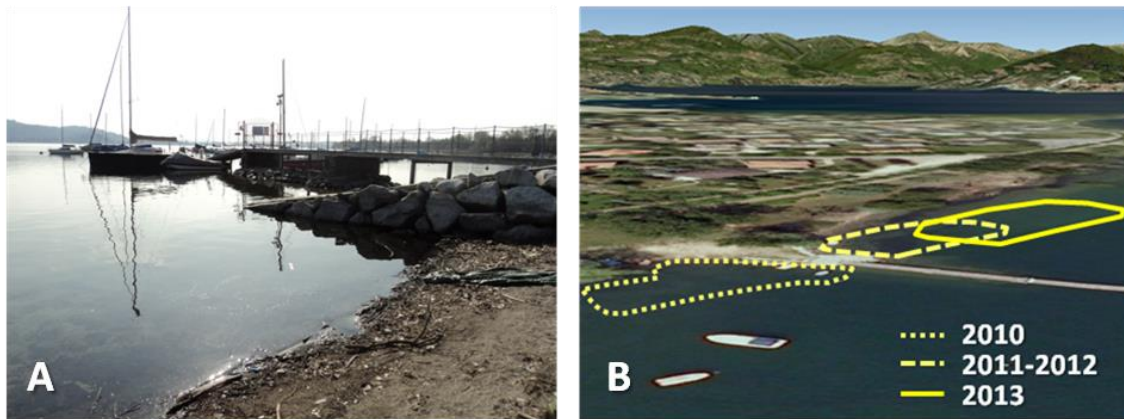


Figure 11: Arona

A - Picture of pier yachting of Arona

B - Google maps image with sampling area highlighted in yellow (different lines define different years)

Arona (Figure 11): sampling site located in the southern part of the village and is characterized by nautical sailing boats and motor boats mooring. Bathing activities are not allowed. The sediment is dark and anoxic, covered with a fine particulate that hide terrestrial leaf litter and different types of wastes. Along the shoreline is also present a water discharge.

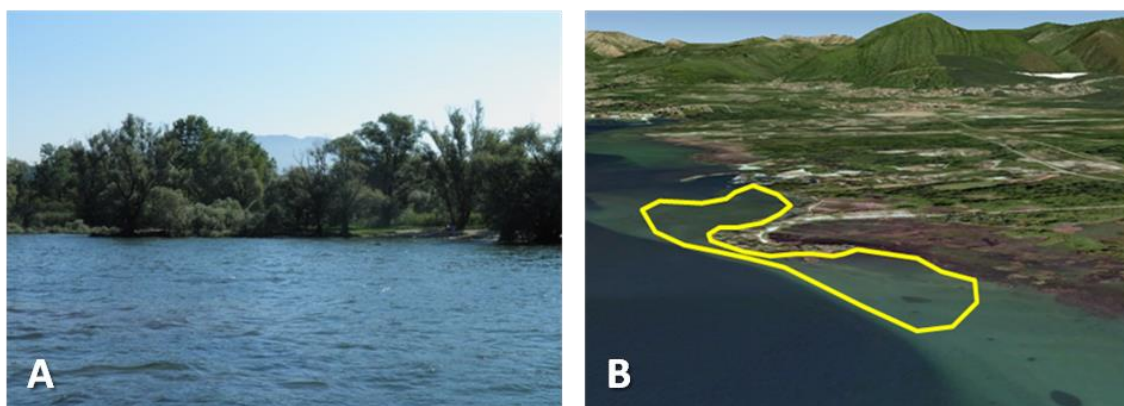


Figure 12: Brebbia

A - Picture of “Sabbie d’oro” beach at Brebbia

B - Google maps image with sampling area highlighted in yellow

Brebbia (Figure 12): relatively undisturbed area, subject of protection (Natura 2000 - SIC IT2010021), with a cane thicket that host a lot of bird species (Tessaro, 2009). The beach, named “Sabbie d’oro” (golden sand) was frequented for bathing in the past, but presently only few presences are recorded during Summer. Substrate is composed by high sediment patchiness, also shore morphology is very variable, with inlets, small islands and gentle slope, offering a multitude of different microhabitats.

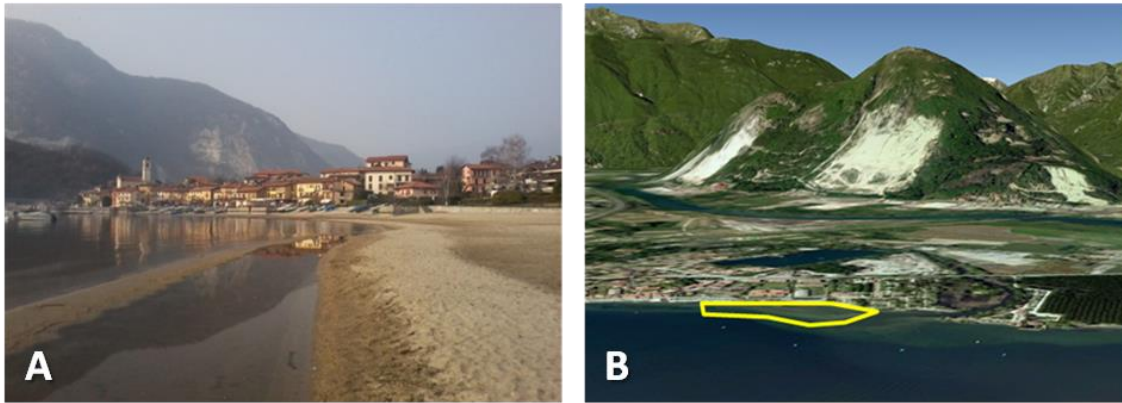


Figure 13: Feriolo
 A - Picture of Feriolo beach and village
 B - Google maps image with sampling area highlighted in yellow

Feriolo (Figure 13): this appreciated beach take the name from the village nearby. During Summer the site is under pressure of recreational activities (boating, bathing, etc.). Beside the beach is located a camping and nearby the Toce river has his outlet. Fine heterogeneous depositions river-mediated forms a gentle slope with sandy bottom.

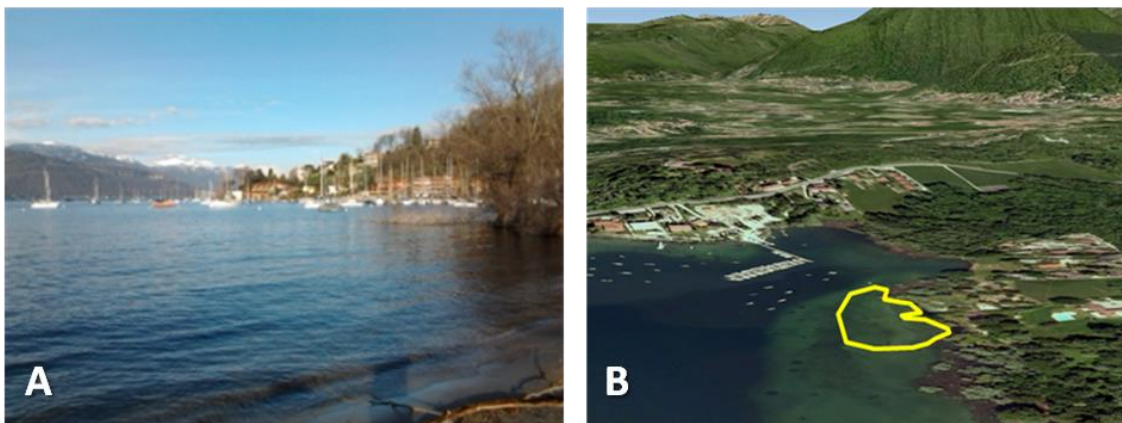


Figure 14: Monvalle
 A - Picture of "Gurè" beach and sailing club at Monvalle
 B - Google maps image with sampling area highlighted in yellow

Monvalle (Figure 14): characterized by a beach and a sailing boating nearby, some spots of cane thicket are present, slope decrease gently and sediment is a patch of different sizes defining different microhabitat, passing from a sandy to pebbly bottom. This site was added during Summer 2011, because before that moment no alien species were recorded.

2.3 *Sampling strategy*

Qualitative sampling are performed to control native mussels population, to check eventually new sites colonized by alien species and to collect organisms for laboratory analysis. In those case a underwater viewer (Aquascope) and a sampling mussels net were used to find and collect molluscs (Figure 15).

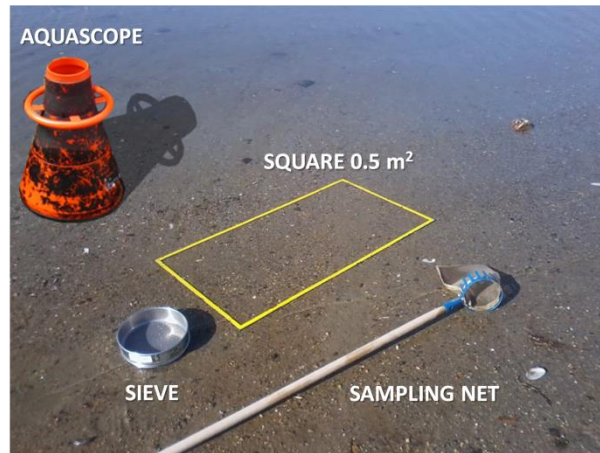


Figure 15: Sampling materials

Quantitative sampling were performed seasonally to investigate *Corbicula* population dynamics, periods of recruitment and mortality (Table 2).

	Angera	Arona	Brebbia	Feriollo	Monvalle
Autumn 2010	✓	✓	✓	✓	
Spring 2011	✓	✓	✓	✓	
Summer 2011	✓	✓	✓	✓	✓
Autumn 2011	✓	✓	✓	✓	✓
Winter 2012	✓	✓		✓	
Spring 2012	✓	✓	✓	✓	✓
Summer 2012	✓	✓	✓	✓	✓
Autumn 2012	✓	✓	✓	✓	✓
Winter 2013	✓	✓	✓	✓	✓
Spring 2013	✓	✓	✓	✓	✓

Samples were collected using different methodology due to water level: when access from the shore was comfortable a molluscs sampling net (mesh size 0.1 mm) has been used to collect sediment for 15-20 cm of depth within a known area delimited with a square of defined surface (0.5, 0.25 or 0.0625 m²), randomly positioned (Figure 15).

With a high water level that risks to compromise the effective operability, a diving team was employed to collect quantitative samples adapting the methodology to subaqueous activities (Figure 16). Sampling strategy was improved also evaluating characteristics of different sites performed an adequate number of replicates in order to represent more realistic situations.

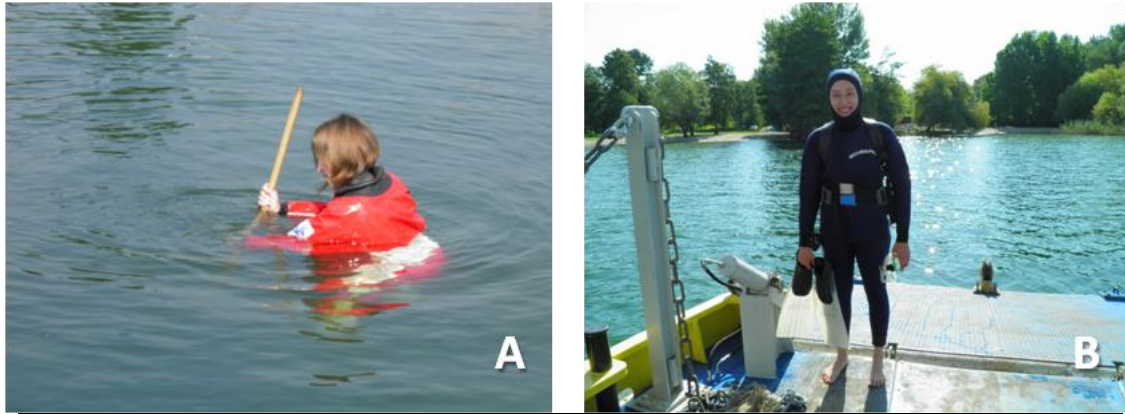


Figure 16: Sampling methodology
 A - Low water level, sediment collection from the shore
 B - Diving activities with high water level

At each site, bivalves were collected by sieving the sediments through a 0.5 mm and 0.1 mm mesh size sieves to isolate big and small animals respectively. Samples were taken at least in triplicates, to cover an area among 1.5 to 5 m² (< 1.5 m water depth, maximum to 50 meters from the shoreline towards the deep).

2.4 *Data and statistical analyses*

2.4.1 *Morphometric characteristics*

2.4.1.1 *Dry weight, organic matter and ashes*

150 specimens collected from the five sampling sites (30 from each locality) were dissected and analysed to determine fresh and dry weight, organic matter and ashes content of soft tissues and shells. Soft tissues from each animal were carefully separated from shells with dissecting instruments (scissors and scalpels). Both, soft tissues and shells were weighed and afterwards were dried individually at 105°C for 24 hours and consequently re-weighed. Ash weight was measured after burning at 450°C for 8 hours.

A regression was applied to the considered variables (length against fresh and dry weight, weight of organic matter and ashes content). Using regression analysis for two independent variables is statistical not correct, but allows comparison among data collected in different sites. Regression ANOVA was performed to test the existence of the coefficients calculated and a Multiple Regression Analysis (ANCOVA) was used to test parallelism of the straight lines.

Parallelism test hypothesizes that the straight lines analysed are similar. The first consideration regards the coefficients of the straight lines:

- Slope comparison: $H_0: \beta_1 = \beta_2$ (no difference between the angular coefficients of straight lines)

If the H_0 is accepted it's possible to proceed with the comparison of the elevation among the straight lines:

- Elevation comparison: $H_0: \alpha_1 = \alpha_2$ (no difference in the position of intersection between the intercepts of straight lines).

2.4.1.2 Biometrical variables

Conventional morphometric characteristics –shell length (L: anterior to posterior margins of the shell), height (H: umbo to gape), and width (W) of each specimen were measured using electronic caliber (to the nearest 0.01 mm) (Figure 17). Smaller specimens (< 5 mm) were measured later in the laboratory under a stereo-microscope (Leica M165C , maximum magnification 120X with eye piece micrometre). Morphometric relationships length-width-height of *C. fluminea* were expressed by regression equation, tested with regression ANOVA and compared with parallelism test.

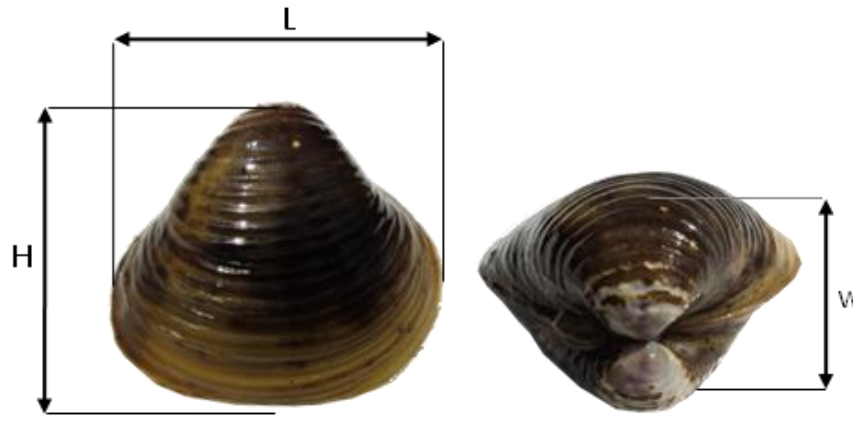


Figure 17: *Corbicula fluminea*
L – total length, H umbo/ventral edge height, W – width shell measurements (mm)

2.4.2 Population density, biomass and their seasonal variations

Densities were evaluated by counting the number of individuals found within a bottom area delimited by the use of a quadrat. Quadrats were positioned randomly to better estimate distributions of bivalves along shoreline. Length-weight relationships were used to estimate standing stock at each site (biomass per square metre).

2.4.3 Population dynamics

C. fluminea length frequency distribution were plotted using 0.5 mm size class intervals (starting from 1.0 mm). Using our data, the estimated potential size range for one, two, and three year old individuals was from 15 to 22.5 mm; from 23.5 to 27 mm, and from 27 to the maximum observed 31 mm respectively (growth constant: 0.65; Cataldo & Boltovskoy, 1998). A Kolmogorov-Smirnov test was used to compare different distributions.

Significative results reject the null hypothesis: compared distributions are the same distributions. This test was performed with two different level of significance with $\alpha = 0.05$ or $\alpha = 0.01$.

2.4.4 Growth rate

2.4.4.1 Cage growth evaluation

An experiment was performed to evaluate growth in semi-natural conditions. Four cages were built to store organisms in a artificial pool at the “Hydrobiological Institute” of CNR – ISE Verbania Pallanza (Figure 18). This pool receive lake water pumped from 20 meters depth twice a day, the surface is approximately 54 m² and depth is 150 cm. Water conserve same chemistry but temperature is different respect littoral (usually warmer).



Figure 18: Pool with lake water

Four cages were prepared, each hosting 40 animals from two sites (Angera and Brebbia) which were measured weekly for one year long. Growth rate was calculated according to Winberg (1971) (Equation 1):

Equation 1: Growth rate
calculation

$$\text{Growth rate} = g = \frac{\log L_t - \log L_{t_0}}{\text{days}}$$

2.4.4.2 *Field growth evaluation*

Growth rates were empirically calculated analysing cohorts displacements over two consecutive sampling at the same site. Size-frequency histograms are often multimodal and each peak corresponds to a cohort, but cohorts are often overlapping making difficult a unequivocal identification especially for larger size classes. Through the application of the Walford method (1946), after the visual identification of the first two cohorts, the modes of the following cohorts were identified.

The methodology of the probability paper proposed by Harding (1949) was applied to improve objectivity of cohorts identification. To this aim cumulative frequencies plots were analysed to detect the inflections which correspond to the cohort limits.

In addition the von Bertalanffy formula was applied on identified cohorts (Equation 2):

Equation 2: von Bertalanffy
growth equation

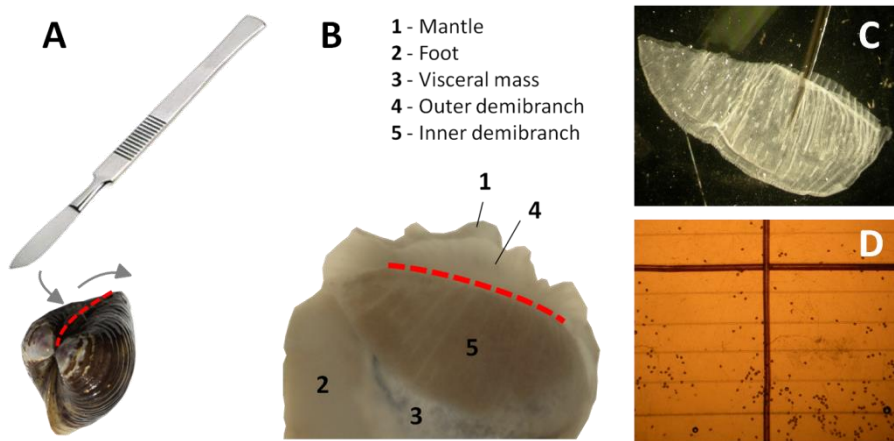
$$l_t = L_\infty(1 - e^{-k(t-t_0)})$$

where t is time, l is shell length (mm), k is the growth rate and L_∞ is the asymptotic length at which growth is zero.

The von Bertalanffy formula was applied using XLSTAT for Excel software (© 1995-2013 Addinsoft)

2.4.5 Reproduction

To estimate reproductive periods and minimum size at maturity of *C. fluminea* in Lake Maggiore hundreds of organisms were stored in the pool (Figure 18) at three different depths (Figure 20). Every week a sub-sample of 10 organisms was dissected to isolate the inner demibranchs (Figure 19) facilitating microscopical inspection to identify early development stages.



A sub-sample (10 animals) was contemporarily collected in field (Feriolo) for a comparison of the reproductive condition of wild and caged animals.

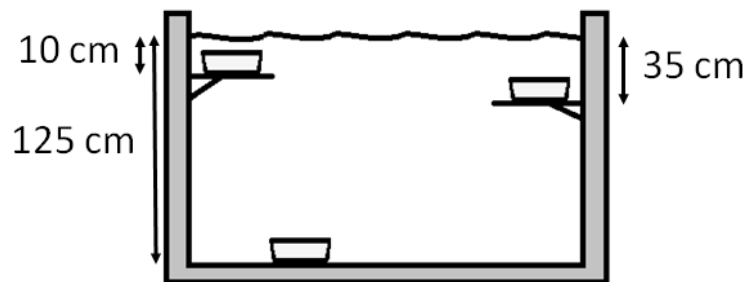


Figure 20: Cage disposition in semi-natural conditions

To estimate fertility, quantitative larval estimation was performed on frozen animals stored after every field sampling. In this case only last stage were preserved (*veliger*), in fact early stage and trochophore were destroyed during the freezing process.

100 claims, when available, were dissected for each sampling. Larvae in inner demibranchs were counted and measured with a eyepiece micrometre. The larval production per square meter was calculated multiplying the percentage of reproductive organisms by the number of larvae per individual.

2.4.6 Clearance rate

Organisms were collected at Brebbia and selected for two different size classes (*Unio elongatulus* about 45 mm and 72 mm, *Corbicula fluminea* 14 mm and 21 mm). This site was chosen for two reasons: the abundance of both species in this site and the data availability of *U. elongatulus* survey of 2004 (Riccardi personal communication).

Different experimental conditions were adopted (initial algae concentration, algae species (*Microcistes* and *Chlorella*), animal density, temperature, night/day/whole day experiments) and clearance rate were measured with FluoroProbe (bbe Moldaenke GmbH, Germany).

2.4.7 Stable isotope analysis

Native and molluscs were collected from sites where they are more abundant (native from Brebbia, *A. woodiana* from Angera and *C. fluminea* from Arona, Brebbia and Feriolo). Analyses were performed on *C. fluminea* juveniles (length < 10 mm) and adults (length > 20 mm). Different organs (mantle, foot and gills) were dissected and pooled to obtain samples representative of an adequate number of animals (> 10). After drying (48 hours at 60°C) samples were powdered in a glass mortar, weighed ($1 \pm 0.2 \mu\text{g}$; Analytical Balance Sartorius GmbH, Germany; sensitivity 0.001 g) and stored in tightly closed aluminium caps. Analyses were performed by G.G. Hatch Isotope Laboratories, University of Ottawa, Faculty of Science (Earth Sciences) using elemental analyser and mass spectrometer (Isotope Cube manufactured by Elementar, Germany - Isotope Ratio Mass Spectrometer (irms): Delta Advantage manufactured by Thermo, Germany). Samples were analyzed following the methodology of G.G. Hatch Isotope Laboratories, University of Ottawa and the data were reported in Delta notation δ . The units are per mill (‰) and defined by the equation 3:

Equation 3: Isotope ratios
equation expressed in δ notation

$$\delta = [(R_x - R_{std})/R_{std}] * 1000$$

where R is the ratio of the abundance of the heavy isotope on the light one, x denotes sample and std is for standard.

$\delta^{15}\text{N}$ were reported as ‰ vs. air and $\delta^{13}\text{C}$ were reported as ‰ vs. V-PDB (Pee Dee Belemnite).

3.1 Morphometric characteristics: *C. fluminea*

3.1.1 Dry weight, organic matter and ashes

Fresh weight is composed by shells for a percentage ranging between 68.6 (Arona) and 79.9 (Angera; Table 3). These percentages are higher if calculated on dry weight (from 92.6 at Brebbia to 95.8 at Angera). Water content in shells is between 4.2 (Angera) and 7.4 (Brebbia), while the quantity of water in soft tissues is remarkably higher (from 82.3 at Monvalle to 85 at Arona). At every site organic matter content is higher than 92% in soft tissues and less than 2.8% in shells. Ashes constitute more than 97% of shell dry weight, while a percentage lower than 8% are present in soft tissues dry weight (from 5.8 % at Brebbia to 7.7 % at Arona).

Table 3: Percentage of shells and soft tissues fresh and dry weight, content of water, organic matter and ashes

Site	% fresh weight		% dry weight			water %		Organic matter		Ashes	
	shell	tissue	shell	tissue	shell	shell	tissue	shell	tissue	shell	Tissue
	total	total	Total	total	tissue	dw	dw	dw	dw	dw	dw
Angera	79.9	20.1	95.8	4.2	22.9	4.8	83.5	2.7	92.7	97.3	7.3
Arona	68.6	31.4	93.2	6.8	13.8	5.1	85.0	2.7	92.3	97.3	7.7
Brebbia	72.7	27.3	92.6	7.4	12.5	4.5	79.7	2.7	94.2	97.3	5.8
Ferriolo	75.6	24.4	94.5	5.5	17.3	4.5	82.8	2.7	93.0	97.3	7.0
Monvalle	71.5	28.5	93.1	6.9	13.6	4.2	82.3	2.8	92.8	97.2	7.2

In the following figures are reported the results of fresh weight (Figure 21), dry weight (Figure 22), organic matter content (Figure 23) and ashes (Figure 24), for both soft tissue and for shell (respectively A and C in the figures). The data are interpolated with a logarithmic curve, with R^2 values higher than 0.9. This type of curve indicates allometric growth.

Generally, observing relations between weight and length it appears that these relations are closer among young smaller size/age organisms of the five sites, while a difference among sites/populations can be observed particularly for larger organisms. However it necessary emphasized that these differences are affected by the lack of data for the higher size classes, in fact, larger specimens are well represented only in sites of Angera and Arona.

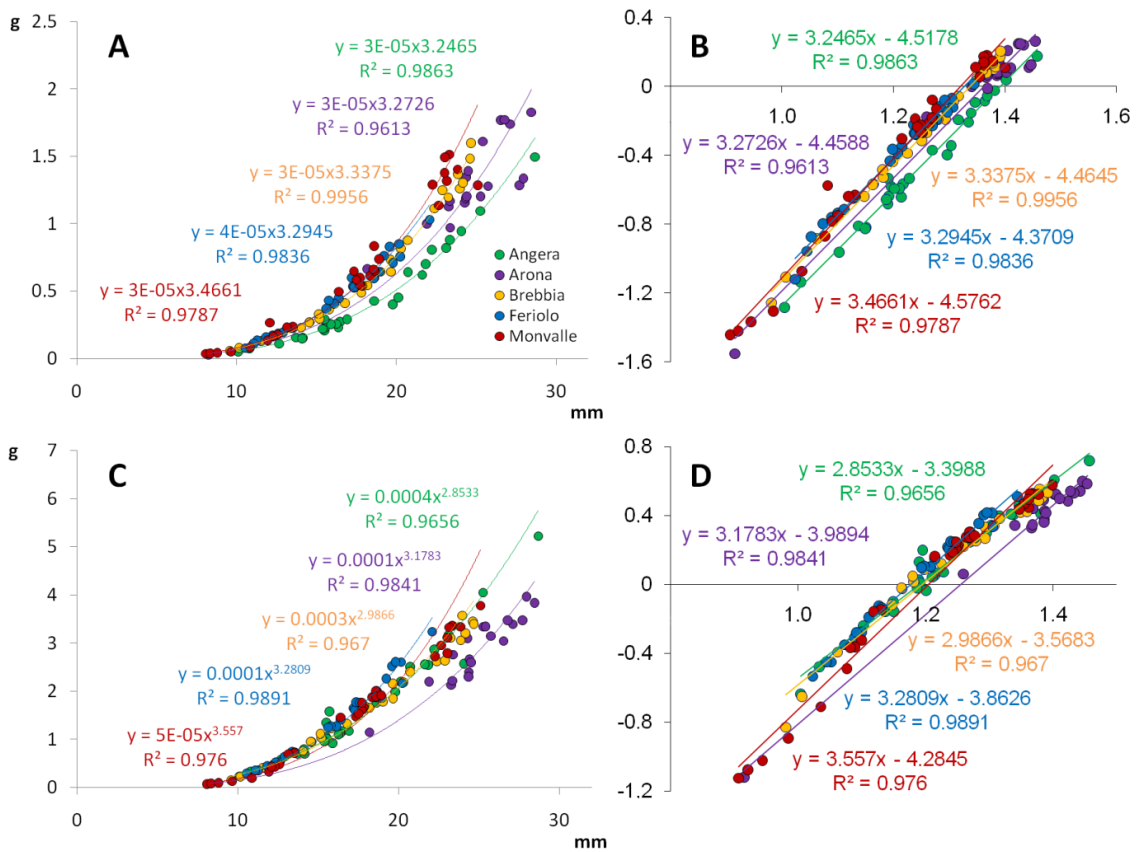


Figure 21: Equations of fresh weight over length at the five stations
 A – Soft tissue fresh weight B – Linearization of soft tissue equations
 C – Shell fresh weight D – Linearization of shell equations

Table 4: Parallelism test on soft tissue and shell equations of figure 21
 Limits $t > 30$: 1.95 ($\alpha=0.05$) *; 2.57 ($\alpha=0.01$) ** and 3.29 ($\alpha=0.001$) ***

Soft tissue					Shell				
Slope	Angera	Arona	Brebbia	Feriolo	Slope	Angera	Arona	Brebbia	Feriolo
Arona	$t_{44} = -0.17$				Arona	$t_{44} = -2.12$ ***			
Brebbia	$t_{51} = -1.04$	$t_{43} = -0.5$			Brebbia	$t_{51} = -0.87$	$t_{43} = 1.26$		
Feriolo	$t_{52} = -0.43$	$t_{44} = -0.13$	$t_{51} = 0.47$		Feriolo	$t_{52} = -3.26$ ***	$t_{44} = -0.9$	$t_{51} = -2.16$ ***	
Monvalle	$t_{54} = 5.78$ ***	$t_{46} = 4.63$ ***	$t_{53} = 6.01$ ***	$t_{54} = 4.88$ ***	Monvalle	$t_{54} = 5.07$ ***	$t_{46} = 4.44$ ***	$t_{53} = 5.35$ ***	$t_{54} = 4.76$ ***
Elevation	Angera	Arona	Brebbia	Feriolo	Elevation	Angera	Arona	Brebbia	Feriolo
Arona	$t_{45} = 3.18$ ***				Arona	-			
Brebbia	$t_{52} = 8.23$ ***	$t_{44} = 3.17$ ***			Brebbia	$t_{52} = 0.19$	$t_{44} = 4.72$ ***		
Feriolo	$t_{53} = 7.51$ ***	$t_{45} = 3.52$ ***	$t_{52} = -28.5$ ***		Feriolo	-	$t_{45} = 8.32$ ***		
Monvalle	-	-	-	-	Monvalle	-	-	-	-

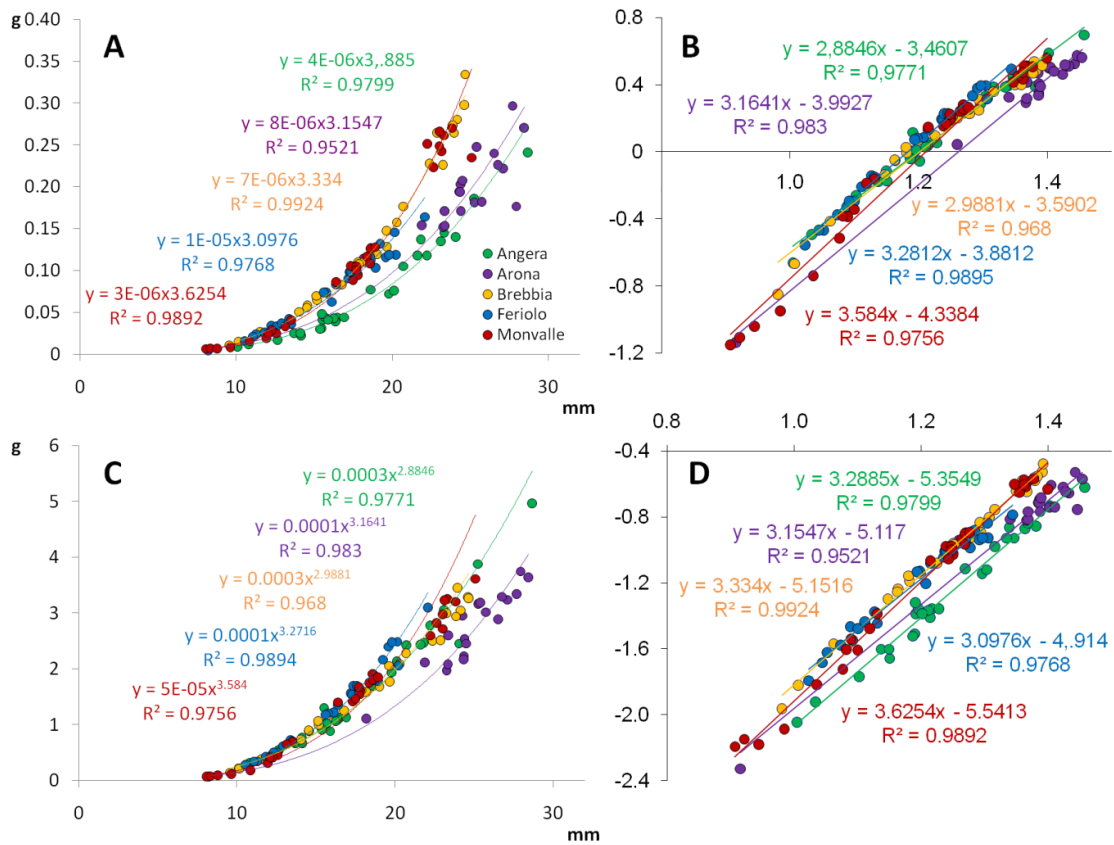


Figure 22: Equations of dry weight over length at the five stations
 A – Soft tissue dry weight B – Linearization of soft tissue equations
 C – Shell dry weight D – Linearization of shell equations

Table 5: Parallelism test on soft tissue and shell equations of figure 22
 Limits $t > 30$: 1.95 ($\alpha=0.05$) *; 2.57 ($\alpha=0.01$) ** and 3.29 ($\alpha=0.001$) ***

	Soft tissue				Shell			
	Angera	Arona	Brebbia	Feriolo	Angera	Arona	Brebbia	Feriolo
Slope								
Arona	$t_{44}=0.75$				Arona	$t_{44}=-2.13$ ***		
Brebbia	$t_{51}=-0.42$	$t_{43}=-1.1$			Brebbia	$t_{51}=-0.74$	$t_{43}=1.16$	
Feriolo	$t_{52}=1.44$ *	$t_{44}=0.31$	$t_{51}=2.19$ ***		Feriolo	$t_{52}=-3.43$ ***	$t_{44}=-0.93$	$t_{51}=-2.11$ ***
Monvalle	$t_{54}=5.65$ ***	$t_{46}=4.35$ ***	$t_{53}=5.81$ ***	$t_{54}=4.49$ ***	Monvalle	$t_{54}=5.1$ ***	$t_{46}=4.4$ ***	$t_{53}=5.32$ ***
Elevation								
Arona	$t_{45}=2.11$ ***				Arona	-		
Brebbia	$t_{52}=8.64$ ***	$t_{44}=5.12$ ***			Brebbia	$t_{52}=0.03$	$t_{44}=4.78$ ***	
Feriolo	-	$t_{45}=3.81$ ***			Feriolo	-	$t_{45}=8.24$ ***	
Monvalle	-	-			Monvalle	-	-	

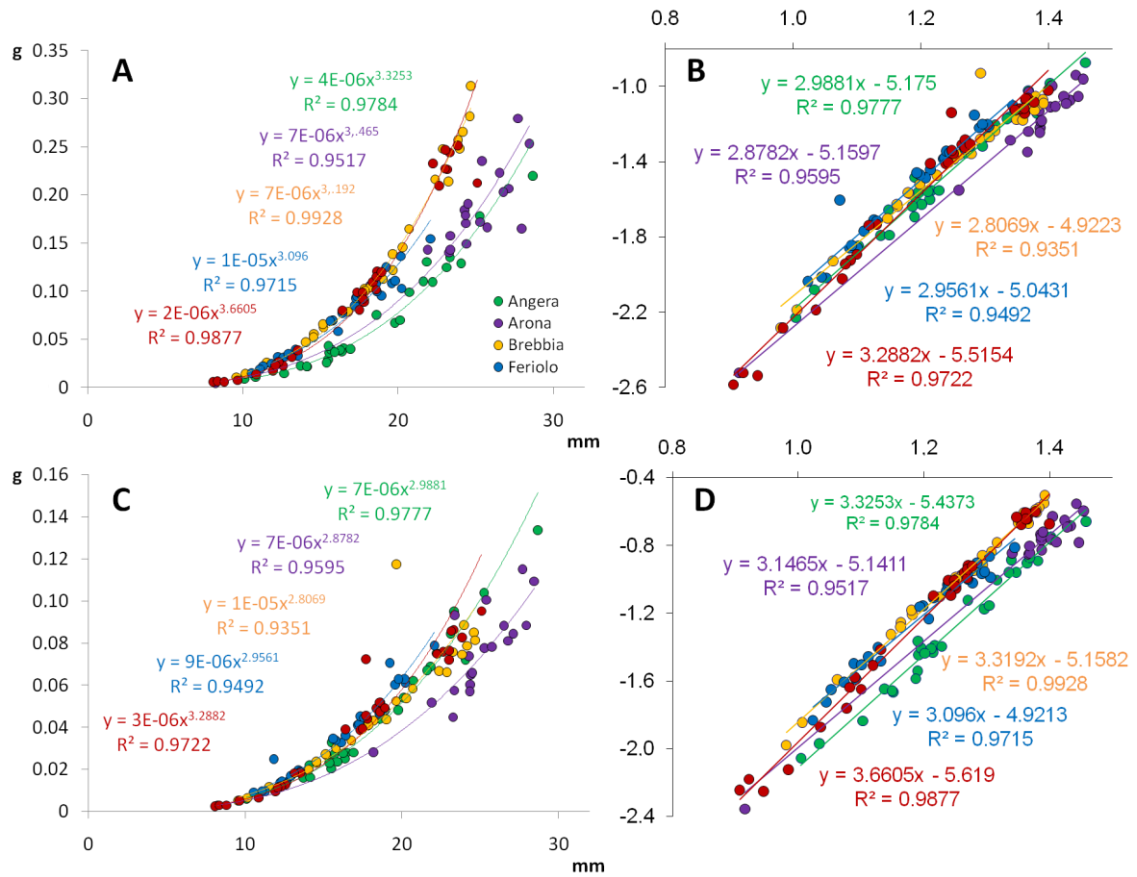


Figure 23: Equations of organic matter over length at the five stations
 A – Soft tissue organic matter B – Linearization of soft tissue equations
 C – Shell organic matter D – Linearization of shell equations

Table 6: Parallelism test on soft tissue and shell equations of figure 23
 Limits $t > 3.0$: 1.95 ($\alpha=0.05$) *; 2.57 ($\alpha=0.01$) ** and 3.29 ($\alpha=0.001$) ***

	Soft tissue				Shell			
	Angera	Arona	Brebbia	Feriolo	Angera	Arona	Brebbia	Feriolo
Slope								
Arona	$t_{44} = 0.98$				$t_{44} = 0.7$			
Brebbia	$t_{51} = 0.05$	$t_{43} = -1.1$			$t_{51} = 1.05$	$t_{43} = 0.34$		
Feriolo	$t_{52} = 1.6^*$	$t_{44} = 0.27$	$t_{51} = 1.95^{***}$		$t_{52} = 0.2$	$t_{44} = -0.41$	$t_{51} = -0.73$	
Monvalle	$t_{54} = 5.65^{***}$	$t_{46} = 4.31^{***}$	$t_{53} = 5.74^{***}$	$t_{54} = 4.45^{***}$	$t_{54} = 5.61^{***}$	$t_{46} = 4.35^{***}$	$t_{53} = 5.38^{***}$	$t_{54} = 4.63^{***}$
Elevation								
Arona	$t_{45} = 2.04^{***}$				$t_{45} = 3.94^{***}$			
Brebbia	$t_{52} = 8.64^{***}$	$t_{44} = 5.28^{***}$			$t_{52} = -2.07^{***}$	$t_{44} = 3.16^{***}$		
Feriolo	-	$t_{45} = 3.72^{***}$	-		$t_{53} = 3.31^{***}$	$t_{45} = 4.47^{***}$	$t_{52} = 1.9$	
Monvalle	-	-	-	-	-	-	-	-

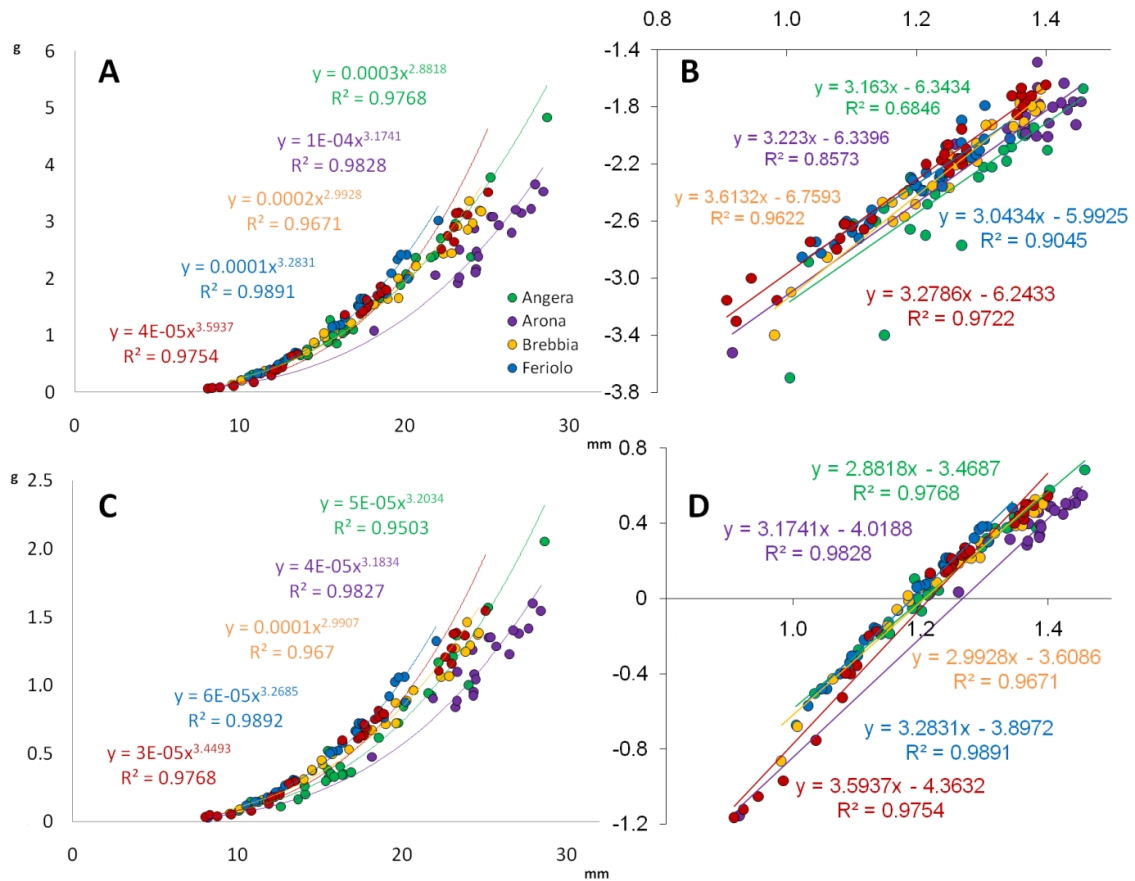


Figure 24: Equations of ash over length at the five stations
 A – Soft tissue organic matter B – Linearization of soft tissue equations
 C – Shell organic matter D – Linearization of shell equations

Table 7: Parallelism test on soft tissue and shell equations of Figure 24
 Limits $t > 3.0$: 1.95 ($\alpha=0.05$) *; 2.57 ($\alpha=0.01$) ** and 3.29 ($\alpha=0.001$) ***

	Soft tissue				Shell				
Slope	Angera	Arona	Brebbia	Feriolo	Slope	Angera	Arona	Brebbia	Feriolo
Arona	$t_{44} = -2.22$ *				Arona	$t_{44} = -0.11$			
Brebbia	$t_{51} = -0.79$	$t_{43} = 1.17$			Brebbia	$t_{51} = -1.02$	$t_{43} = -1.25$		
Feriolo	$t_{52} = -3.52$ ***	$t_{44} = -0.94$	$t_{51} = -2.13$ *		Feriolo	$t_{52} = 0.24$	$t_{44} = -0.51$	$t_{51} = 2.39$ *	
Monvalle	$t_{54} = 5.08$ ***	$t_{46} = 4.4$ ***	$t_{53} = 5.31$ ***	$t_{54} = 4.72$ ***	Monvalle	$t_{54} = 5.44$ ***	$t_{46} = 4.68$ ***	$t_{53} = 6.65$ ***	$t_{54} = 4.72$ ***
Elevation	Angera	Arona	Brebbia	Feriolo	Elevation	Angera	Arona	Brebbia	Feriolo
Arona	$t_{45} = 5.24$ ***				Arona	$t_{45} = 0.83$			
Brebbia	$t_{52} = 0.1$	$t_{44} = 4.72$ ***			Brebbia	$t_{52} = 1.49$	$t_{44} = 1.6$		
Feriolo	-	$t_{45} = 8.17$ ***	-		Feriolo	$t_{53} = 1.72$	$t_{45} = 1.68$	-	
Monvalle	-	-	-	-	Monvalle	-	-	-	-

Linearization (Figures 21 to 24 B and D) and parallelism test (Tables from 4 to 7) performed demonstrate that there are significant differences (ANCOVA $p < 0.005$: Table 8) among straight lines of the five sites. Differences are higher comparing straight lines of shell weight over length ($p < 0.005$), while soft tissue differences are less significant ($p < 0.025$).

Soft tissue	F	DF num	DF den	P
Fresh weight	54.45	123	4	0.005
Dry weight	59.83	123	4	0.005
Organic matter	29.96	123	4	0.005
Ashes	13.35	123	4	0.025

Shell	F	DF num	DF den	P
Fresh weight	71.69	123	4	0.005
Dry weight	126.07	123	4	0.005
Organic matter	123.85	123	4	0.005
Ashes	59.32	123	4	0.005

Parallelism test is applied comparing each straight line with the others (Tables from 4 to 7) identifying similarities between straight lines of Angera and Brebbia (considering straight lines of fresh weight, dry weight and ashes of soft tissue over length) and between Brebbia and Feriolo (comparing straight lines of shell organic matter). Shell ash content straight lines are significant similar in most of the comparisons, with some exceptions (Brebbia-Feriolo and Monvalle respect all others straight lines). Higher values of shell ashes content are recorded for organisms collected at Feriolo and Monvalle, while organisms of Arona presents the lowest values.

3.1.2 Biometrical variables

A highly significant linear regression (ANOVA $p < 0.005$), of height and width over length and of height over width is highlighted (Figure 25).

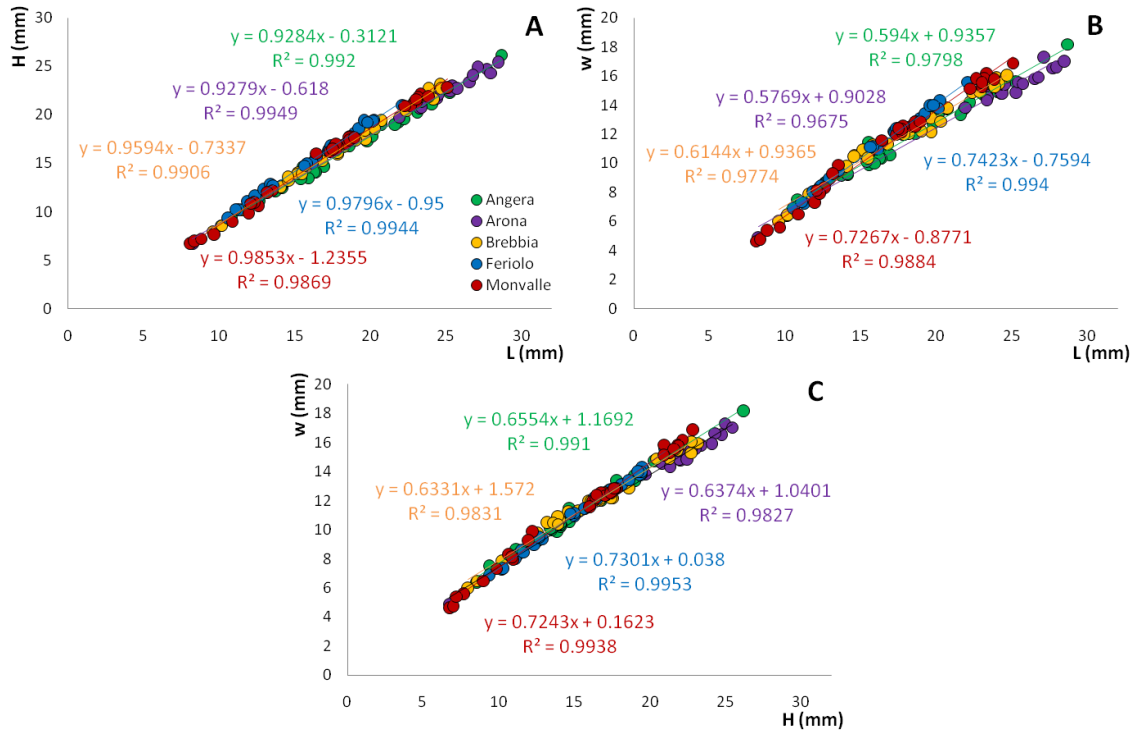


Figure 25: Regression analysis of selected parameters of *C. fluminea*

A - Relation between L/h (length and height in mm)

B - Relation between L/W (length and width in mm)

C - Relation between L/h (height and width in mm)

Applying parallelism test significant difference emerge among straight lines of the five sampling sites (ANCOVA $p < 0.005$; Table 9).

	F	DF num	DF den	P
L/H	52.53	140	4	0.005
L/W	65.17	140	4	0.005
H/W	27.65	140	4	0.005

The test applied to the single couple of straight lines (Table 10) highlights similarities between the following comparisons: Angera-Arona (for straight lines of height over length); Angera-Brebbia, Angera-Feriolo, Angera-Monvalle and Feriolo-Monvalle (for straight lines of H/W).

Table 10: Parallelism test on biometrical variables (Figure 25)
Limits $t > 30$: 1.95 ($\alpha=0.05$) *; 2.57 ($\alpha=0.01$) ** and 3.29 ($\alpha=0.001$) ***

Length-Height					Length-Width				
Slope	Angera	Arona	Brebbia	Feriolio	Slope	Angera	Arona	Brebbia	Feriolio
Arona	$t_{44} = -0.06$				Arona	$t_{44} = 0.59$			
Brebbia	$t_{52} = -2.71$ ***	$t_{44} = -2.35$ ***			Brebbia	$t_{52} = -0.82$	$t_{44} = -1.24$		
Feriolio	$t_{51} = 4.74$ ***	$t_{43} = -4.18$ ***	$t_{51} = -1.93$ **		Feriolio	$t_{51} = -6.53$ ***	$t_{43} = -6.13$ ***	$t_{51} = -5.3$ ***	
Monvalle	$t_{54} = -4.22$ ***	$t_{46} = -3.54$ ***	$t_{54} = -1.43$ *	$t_{53} = 0.52$	Monvalle	$t_{54} = -5.9$ ***	$t_{46} = -5.38$ ***	$t_{54} = -4.77$ ***	$t_{53} = 0.69$
Elevation	Angera	Arona	Brebbia	Feriolio	Elevation	Angera	Arona	Brebbia	Feriolio
Arona	$t_{45} = -0.9$				Arona	$t_{45} = 3.23$ **			
Brebbia	-	-	-		Brebbia	$t_{53} = -3.42$ ***	$t_{45} = -6.51$ ***		
Feriolio	-	-	-		Feriolio	-	-	-	
Monvalle	-	-	-	$t_{54} = 5.99$ ***	Monvalle	-	-	-	$t_{54} = 4.25$ ***

Height-Width				
Slope	Angera	Arona	Brebbia	Feriolio
Arona	$t_{44} = 0.25$			
Brebbia	$t_{52} = 1.14$	$t_{44} = 0.79$		
Feriolio	$t_{51} = -0.04$	$t_{43} = -0.5$	$t_{51} = -26.16$ ***	
Monvalle	$t_{54} = 0.07$	$t_{46} = -0.74$	$t_{54} = -4.95$ ***	$t_{53} = -0.02$
Elevation	Angera	Arona	Brebbia	Feriolio
Arona	$t_{45} = 3.44$ ***			
Brebbia	$t_{53} = 0.49$	$t_{45} = -3.24$ ***		
Feriolio	$t_{52} = -0.57$	$t_{44} = -5.2$ ***	-	
Monvalle	$t_{55} = -0.3$	$t_{47} = -3.45$ ***	-	$t_{54} = 0.12$

3.2 Population density, biomass and their seasonal variations

A very large variation in population density was observed at different sites since the first detection of Asian clam in 2010 (Table 11 and Figure 28). The highest values were detected in the eastern sites of Angera and Brebbia, with values twofold those measured in Feriolio and one order of magnitude higher than in Arona. Generally higher values of density are recorded in Summer and Autumn.

Table 11: Mean density (ind.m²) recorded seasonally at the five sampling sites

Site	AUT	SPR	SUM	AUT	WIN	SPR	SUM	AUT	WIN	SPR
	2010	2011	2011	2011	2012	2012	2012	2012	2013	2013
Angera	1222	1652	676	1247	517	707	865	35	58	18
Arona	87	68	83	90	36	44	100		66	136
Brebbia	1249	181	264	981		2418	556	946	130	235
Feriolio	655	171	398	1148	347	438	311	303	163	136
Monvalle			37	47		61	228	133	48	72

The mean density of population presents a maximum of 2418 ind.m⁻² in Brebbia, while minimum values were recorded in Arona and Monvalle (respectively 90 ind.m⁻² e 40 ind.m⁻²). Angera presented high density until Autumn 2012, with value never lower than 500 ind.m⁻² and a peak of 1652 ind.m⁻² (Spring 2011). In late Summer 2012, water level of the lake decreases and the concomitant increases of water temperature in the littoral area cause a great mortality of *Corbicula* with a reduction of the density to 35 ind.m⁻². In the others sites slopes are more soft and only in Angera the mass mortality is observed.

Arona shows a different situation: density was always lower than 100 ind.m⁻², with a mean value of 71 ind.m⁻².

Brebbia presents the greater variation observed: lower values are recorded in Spring 2011 and Winter (181 and 130 ind.m⁻² respectively) and values near 1000 ind.m⁻² in Autumn 2010, Autumn 2011, Spring 2012 and Autumn 2012 (1249, 981, 2418 and 946 ind.m⁻²). Feriolo population presents lower densities than Angera and Brebbia populations, with the maximum in Autumn 2011 (1148 ind.m⁻²) and the minimum in Spring 2013 (136 ind.m⁻²). Monvalle is the last colonized site among the ones of this study. *C. fluminea* presences, recorded just in Summer 2011, provide the opportunity to follow the initial phase of colonization, called *lag* phase. At the first sampling density was about 37 ind.m⁻², this datum increases up to 228 ind.m⁻² after first year of sampling (from Summer 2011 to Summer 2012).

Table 12: Mean shell densities (ind.m⁻²) recorded seasonally at the five sampling sites

Site	SPR 2011	SUM 2011	AUT 2011	WIN 2012	SPR 2012	SUM 2012	AUT 2012	WIN 2013	SPR 2013
Angera	264	23		996	611	1324	2635	824	428
Arona	41	20	59	16	239	220	226	477	112
Brebbia	12	73	188		280	38	122	77	439
Feriolo	92	22	58	28	69	274	13	257	96
Monvalle					8	41	114	11	10

For a better understanding of the population density variations in different sites, in addition to living organisms, were harvested and counted the shells deposited after the death of molluscs. For Angera during 2012, in addition to the high density of organisms collected, are also observed high densities of the shells (starting from 611 ind.m⁻² in Spring and arriving to 2635 ind.m⁻² in the following Autumn, Table 12 and Figure 28).

Arona presents a variable situation with peaks in Autumn 2011, Spring 2012 and Winter 2013 (59, 239 and 477 ind.m⁻² respectively).

At Feriolo and Brebbia usually shells densities do not exceed 200 ind.m⁻², except in few occasions as Spring 2012 (Brebbia 280 ind.m⁻²), Summer 2012 (Feriolo 274 ind.m⁻²), Winter 2013 (Feriolo 257 ind.m⁻²) and Spring 2013 (Brebbia 439 ind.m⁻²).

Monvalle is the site where lower shells densities were recorded in comparison to the others sites (maximum mean value 114 ind.m⁻² in Autumn 2012).

In table 13 are reported mean lengths of alive organisms collected during e3ah sampling.

In Autumn 2010, the highest mean shell length was recorded in Arona (15 mm) and the lowest in Feriolo (ca. 7 mm on mean); intermediate sizes (averaging 12 mm length) were measured in Brebbia and Angera (Table 13 and Figure 26).

An increasing maximum shell length was measured in the four stations according to the order: Feriolo>Brebbia>Arona>Angera.

Specimens with a shell length >25 mm were only found in the sites of Arona and Angera, while the maximum length decreased a few kilometres northward. In fact at Brebbia maximum length was about 23.7 mm and the lowest (16.7 mm) in the northernmost station (Feriolo).

Table 13: Mean organisms length (mm) recorded seasonally at the five sampling sites

Site	AUT 2010	SPR 2011	SUM 2011	AUT 2011	WIN 2012	SPR 2012	SUM 2012	AUT 2012	WIN 2013	SPR 2013
Angera	11.7	15.4	15.0	19.7	19.8	19.7	21.7	21.1	16.8	16.4
Arona	14.9	12.7	19.1	21.1	23.1	22.6	23.1		8.7	22.2
Brescia	12.0	12.6	16.3	16.3		17.4	16.8	19.2	18.0	20.2
Feriolo	7.4	6.2	11.0	8.9	12.5	11.0	12.7	17.4	10.6	16.4
Monvalle			5.5	10.2		11.9	14.3	9.3	9.8	9.2

An increasing trend in the size of individuals is evident in Brescia, which are also represented size classes larger than 25 mm, already in 2011, while only in 2012 the same size class appears in the organisms of Feriolo. In the spring of 2013, the maximum length found in Monvalle is still less than 25 mm.

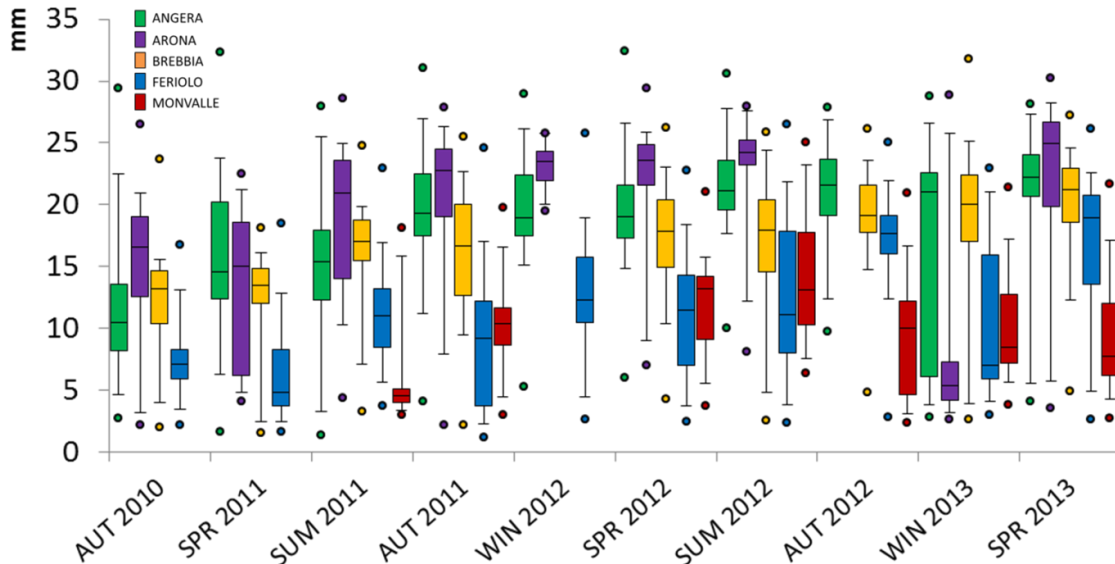


Figure 26: Seasonal variation of length in the five stations

Box plot: 1st quartile - median - 3rd quartile; Whiskers: 5th percentile - 95th percentile; Outliers: minimum and maximum

The empty shells found during sampling follow a similar trend, with an increase of maximum length sampling by sampling (Table 14 and Figure 27). In Spring 2011 the maximum length of the shells is recorded in Angera (maximum length > 25mm); following year shells of similar size dimensions are found in Arona and later in Brescia. Feriolo is characterized by shells of smaller dimensions, reaching length of 25 mm just in Autumn 2012.

A Monvalle were collected only the shells belonging to the smaller size classes.

Site	SPR 2011	SUM 2011	AUT 2011	WIN 2012	SPR 2012	SUM 2012	AUT 2012	WIN 2013	SPR 2013
Angera	15.4	6.5		18.2	17.3	18.7	19.2	19.9	19.5
Arona	11.3	15.9	19.1	17.0	18.9	20.9	19.4	21.2	21.2
Brescia	11.1	11.1	11.8		13.5	8.9	16.8	16.0	15.5
Feriolo	5.2	7.9	9.5	12.3	8.3	11.3	14.2	9.1	12.0
Monvalle					10.6		7.5	8.6	7.3

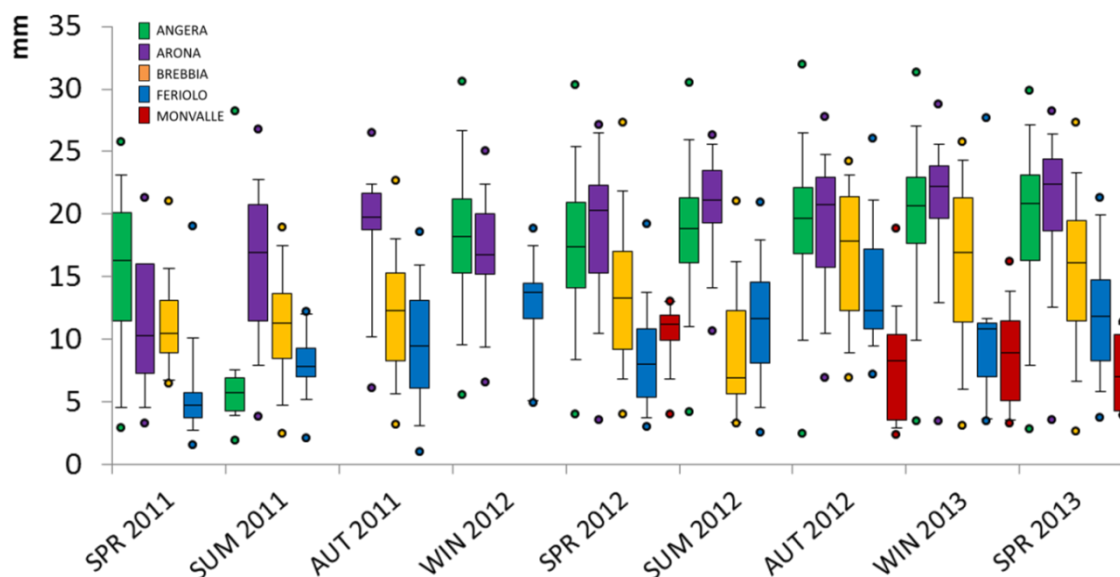


Figure 27: Seasonal variation of empty shell length in the five stations
 Box plot: 1st quartile - median - 3rd quartile; Whiskers: 5th percentile - 95th percentile;
 Outliers: minimum and maximum

The mean biomass estimated for the populations in the five sites is reported in table 15. The abundant populations of Angera and Brescia present also the highest biomass (maximum mean values 2075 g m⁻² and 2453 g m⁻² respectively; Figure 28).

In spite of a relatively high density of clams, Feriolo population showed the lowest biomass (41 g m⁻²), while Arona, with the lowest clams density, had a biomass (103 g m⁻²) more than twice higher than Feriolo.

Site	AUT 2010	SPR 2011	SUM 2011	AUT 2011	WIN 2012	SPR 2012	SUM 2012	AUT 2012	WIN 2013	SPR 2013
Angera	741.1	1825.5	758.4	2420.7	984.2	1328.4	2074.5	80.3	101.0	39.1
Arona	68.5	41.9	126.0	177.1	81.9	116.5	248.3		31.9	330.3
Brescia	821.2	132.4	395.8	1630.8		2453.1	1051.2	2229.5	316.4	757.4
Feriolo	74.3	17.5	146.6	346.2	195.3	192.4	245.0	399.2	90.6	446.1
Monvalle			4.1	15.9		31.7	267.1	42.4	23.1	25.0

Biomasses of empty shells collected in a square meter are reported in table 16. The densities and biomasses are higher in Angera and Arona, due to the high number of shells, but also to the size of the animals that constitute these populations.

The sediments of Brebbia and Feriolo are composed of a lower weight in empty shells, with values that rarely exceed 200 g m⁻² (with the maximum in Spring 2013 at Brebbia with 641.5 g m⁻²).

Monvalle has the lowest values (with maximum of 18 g m⁻² in Autumn 2012) due to the low density and the presence of small size animals.

Table 16: Mean empty shell biomass (g m⁻²) calculated seasonally at the five sampling sites

Site	SPR 2011	SUM 2011	AUT 2011	WIN 2012	SPR 2012	SUM 2012	AUT 2012	WIN 2013	SPR 2013
Angera	279.9	5.7		1556.6	837.2	895.8	4632.8	1609.0	818.5
Arona	14.2	17.5	69.7	15.8	336.9	356.0	297.2	829.6	199.8
Brebbia	6.1	39.1	101.0		274.7	15.9	206.3	129.7	641.5
Feriolo	4.5	2.4	16.5	111.7	11.7	111.0	10.6	437.8	213.3
Monvalle					2.2	15.4	18.0	1.6	1.1

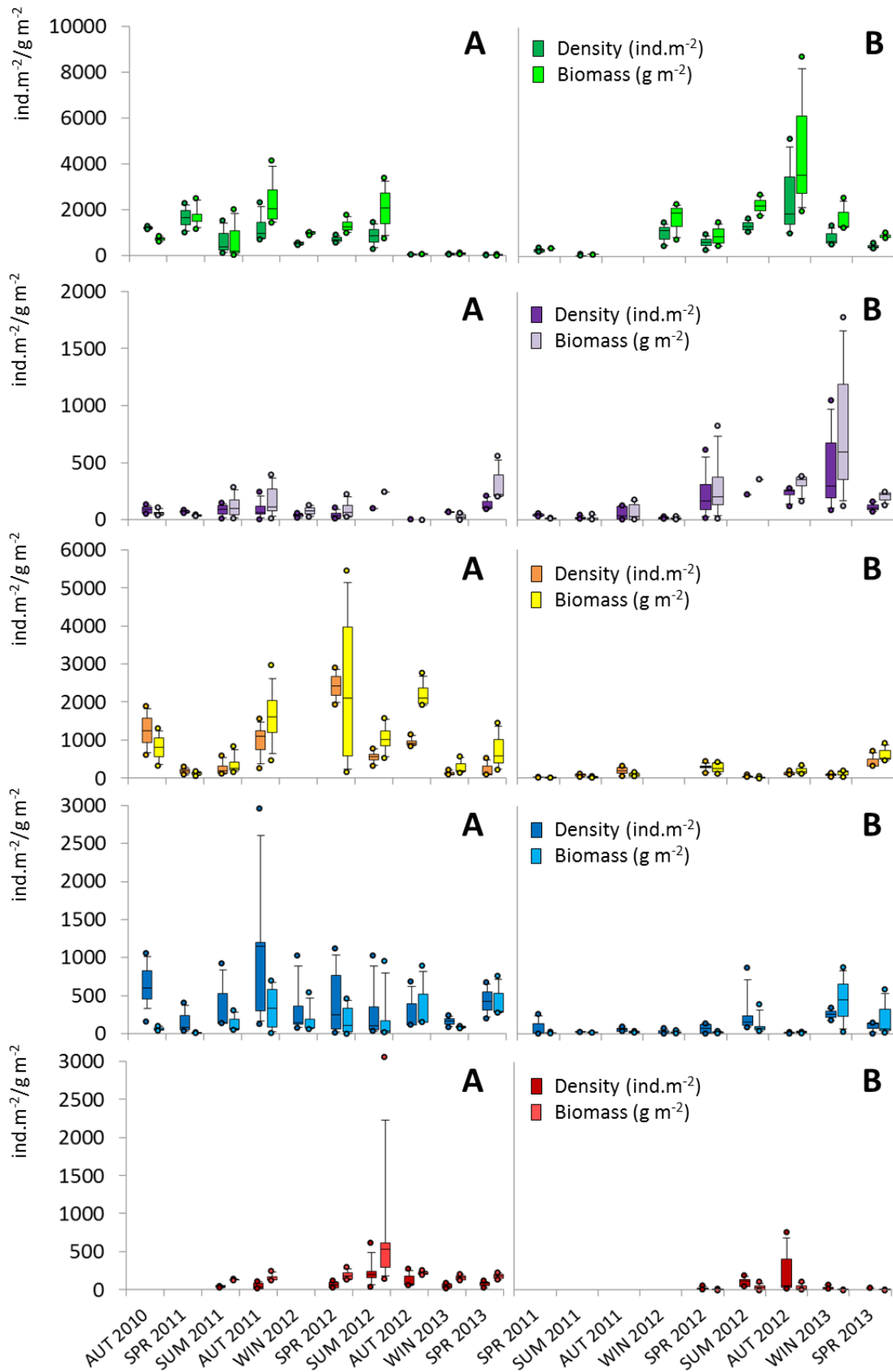


Figure 28: Seasonal variation of density and biomass of *C. fluminea* at the five sampling sites
 A – Alive organisms
 B – Empty shells
 y-axis maximum refer to maximum value observed for each site
 Box plot: 1st quartile - median - 3rd quartile; Whiskers: 5th percentile - 95th percentile; Outliers: minimum and maximum

The density of living specimens and the shells was measured at different depths, to evaluate a depth limit and/or an optimum for the populations currently in the lake. *Corbicula* is present within 9 m depth, but with higher density where seabed has a depth less than 2 m (Figure 29).

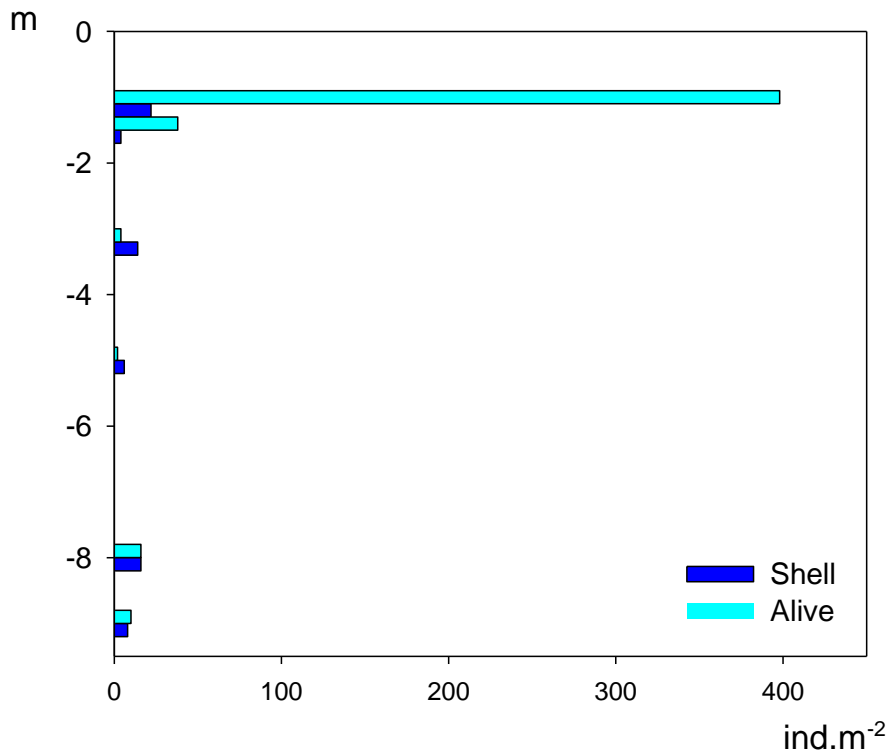


Figure 29: Relation between density and depth of *C. fluminea*

3.3 Population dynamics

Already in Autumn 2010 are highlighted differences in the size structure of the five populations (Figure 30).

Arona population is composed mainly by organisms of 1 year old or less (57.89 % and 41.42% respectively); similar situation is recorded in Angera, with higher proportion of animals born during the year (80.69 %) (Table 17).

Feriolo and Brebbia populations are represented by more than 90 % of juveniles of the year. Angera, Arona and Brebbia appear as asymmetric multimodal mode of size distribution, while Feriolo shows a unimodal distribution. An increasing trend in size appears following the order: Feriolo < Brebbia < Angera < Arona.

The number of cohorts observed in the sampled populations is different between stations; at Angera in Autumn 2010 the three cohorts are identified (< 6 mm, from 7 to 15 mm, > 19 mm corresponding to 80.7 %, 17 % and 2.3 % respectively of total density). The growth of these three cohorts is evident in 2011, with the admission of a new cohort in Summer. The

year 2012 is characterized by two main cohorts consist of animals of one and two years old contributing to more than 70% of the total density detected. A new admitted cohort appears in Winter 2013 (approximately 33 % of the density).

Also at Arona three cohorts are distinguishable (< 5 mm, from 9 to 12 mm, >13 mm individuals) and their growth is evident during 2011. At the beginning of 2012 (Winter) the population is constituted for 51.9 % and 48.1 % by one and two years old animals respectively. New cohort admission is observed in Winter 2013, where the population density is composed for 77.3 % by new born organisms.

At Brebbia two main cohorts are defined in Autumn 2010 (< 6 mm and from 8 to 17 mm), the first is a cohort just admitted and the second is composed mostly by animals born in the year. During 2011 these cohorts continuous to growth. In 2012 a new admitted cohorts is observed in Summer (about 8% of density), while a more abundant event is recorded Winter 2013 (around 15 % of total sampled density).

Feriolò is composed by a unique cohort at the first sampling (< 15 mm about 99 % of density), new cohorts admission appear more frequent than other sites (53 % of density in Spring 2011, 33 % in Autumn 2011 and 15 % in Winter 2013). In any case the presence of organisms with length below 5 mm is almost constant in all samples (except in Autumn 2012).

Monvalle first sample happen in Summer 2012 and population is composed for 93 % by organisms born during the year. The percentage of organisms born during the year remain the highest during following sampling, with value greater than 90 % until Winter 2013, with the exception of Summer 2012 (55 %). After the percentage is reduced to about 86-87% and the percentage of animals with one year of age increases. New cohort admissions seem happened in Autumn 2011 and Winter 2013.

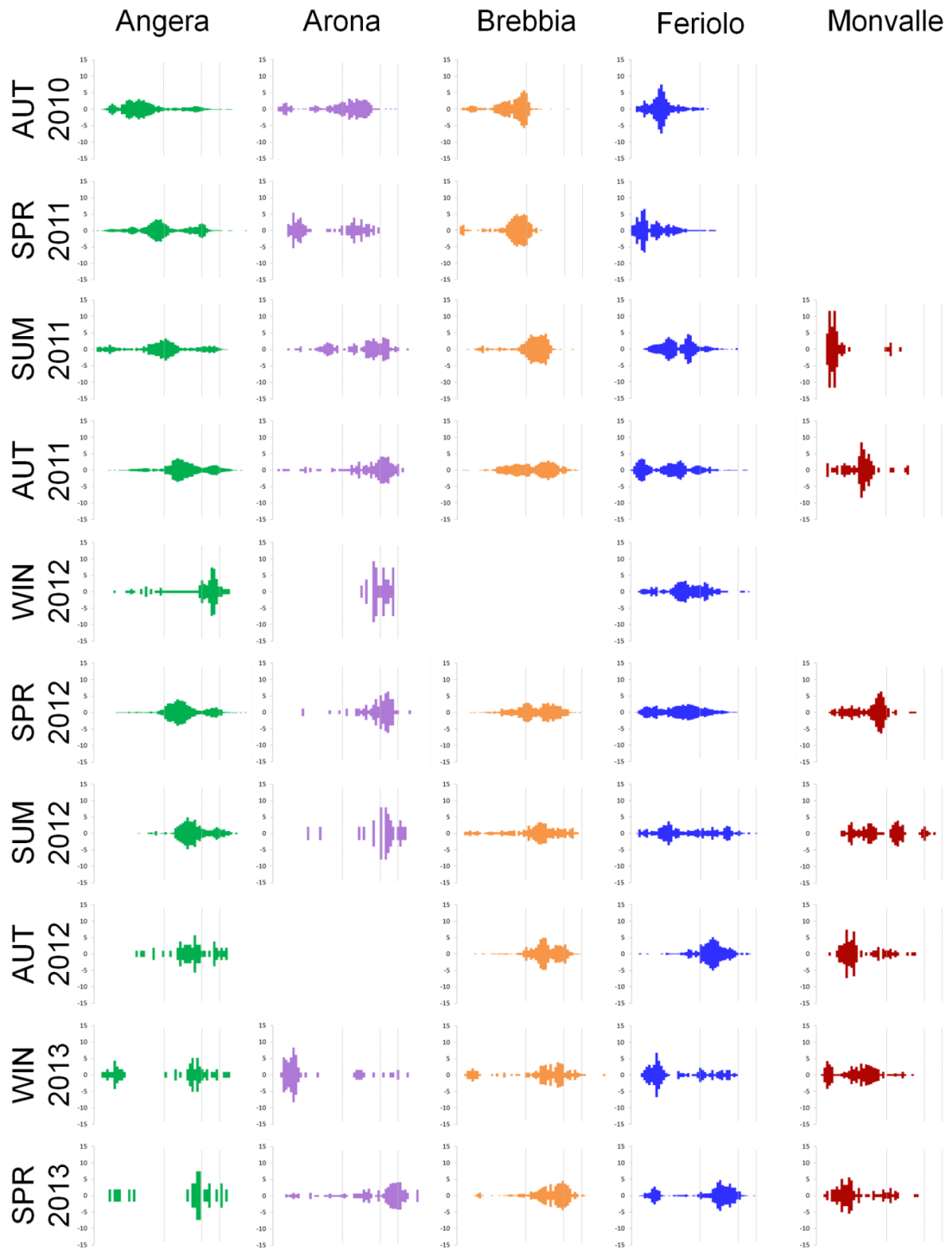


Figure 30: Population structure monitored at the five sampling sites
 Percentage frequencies distributed in size classes of 0.5 mm width
 Vertical lines indicate approximated age of cohorts

Table 17: Percentage age distribution of sampled populations
Conditional formatting: from red (100%) to green (0%)

	AGE	AUT 10	SPR 11	SUM 11	AUT 11	WIN 12	SPR 12	SUM 12	AUT 12	WIN 13	SPR 13
ANGERA	< 1 Year old	80.69	60.53	51.58	11.87	15.50	7.36	2.89	11.32	32.76	22.22
	1 Year old	17.02	32.38	36.26	66.48	21.71	72.77	71.68	62.26	50.00	48.15
	2 Years old	1.88	6.72	11.56	16.68	55.04	16.41	17.11	16.98	12.07	18.52
	3 Years old	0.41	0.36	0.59	4.97	7.75	3.47	8.32	9.43	5.17	11.11
ARONA	< 1 Year old	41.42	52.94	27.11	11.39	0.00	4.60	8.00	0.00	77.27	4.60
	1 Year old	57.89	47.06	47.59	48.73	51.85	44.83	32.00	0.00	13.64	44.83
	2 Years old	0.69	0.00	24.70	38.61	48.15	49.43	44.00	0.00	7.58	49.43
	3 Years old	0.00	0.00	0.60	1.27	0.00	1.15	16.00	0.00	1.52	1.15
BREBBIA	< 1 Year old	93.76	88.37	25.05	41.56		33.88	28.06	10.68	21.54	9.65
	1 Year old	6.08	11.63	74.76	56.62		63.08	63.67	83.83	64.62	74.75
	2 Years old	0.16	0.00	0.19	1.82		3.04	8.27	5.50	12.82	15.35
	3 Years old	0.00	0.00	0.00	0.00		0.00	0.00	0.00	1.03	0.25
FERIOLO	< 1 Year old	98.98	98.05	90.28	90.93	73.49	82.63	63.84	20.48	72.39	28.51
	1 Year old	1.02	1.95	9.72	8.71	25.07	17.37	34.62	77.97	27.61	70.42
	2 Years old	0.00	0.00	0.00	0.36	1.44	0.00	1.54	1.54	0.00	1.08
	3 Years old	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MONVALLE	< 1 Year old			93.15	92.96		93.37	55.26	93.50	87.37	86.11
	1 Year old			6.85	7.04		6.63	41.23	6.50	12.63	13.89
	2 Years old			0.00	0.00		0.00	3.51	0.00	0.00	0.00
	3 Years old			0.00	0.00		0.00	0.00	0.00	0.00	0.00

Observing figure 30, it's possible to imagine the times of colonization, for which the same considerations for the lengths of organisms are valid: Monvalle<Feriolo<Brebbia<Angera<Arona. This observation is confirmed by analysis of the size structure of the populations.

The Kolmogorov-Smirnov test identifies significant similarities between the populations frequency distributions of Angera and Arona (α 0.05 in 9 comparisons and α 0.01 in 4 comparisons), these sites have been colonized before the other sampling sites and the growth of the animals reflects this situation (Figure 31).

The similarities between the Arona population (Spring 2012) and populations of Brebbia and Feriolo are due to the reduced number of organisms sampled at Arona due to the high mortality in this population which appears to be subject.

There are some interesting similarities found between Feriolo and Monvalle (α 0.05 in 3 comparisons and α 0.01 in 3 comparisons), the results indicate that these frequency distributions are composed mainly by small organisms.

The changes in the structure and size of the population are investigate for each site. At Feriolo no distribution is similar to another, while in Brebbia the distribution found in Spring 2013 is similar to one of the previous sampling (Winter 2013 α 0.05).

The frequency distribution of Monvalle is similar in 3 comparisons (Autumn 2011-2012 and Winter-Spring 2013 with α 0.05, Autumn 2012-Winter 2013, with α 0.01).

The frequency distributions of Angera and Arona are significantly similar in a greater number of comparisons respect the other sites (6 comparisons with α 0.05 and 4 with α 0.01 for Angera ; 3 comparisons with α 0.05 and 6 with α 0.01 for Arona).

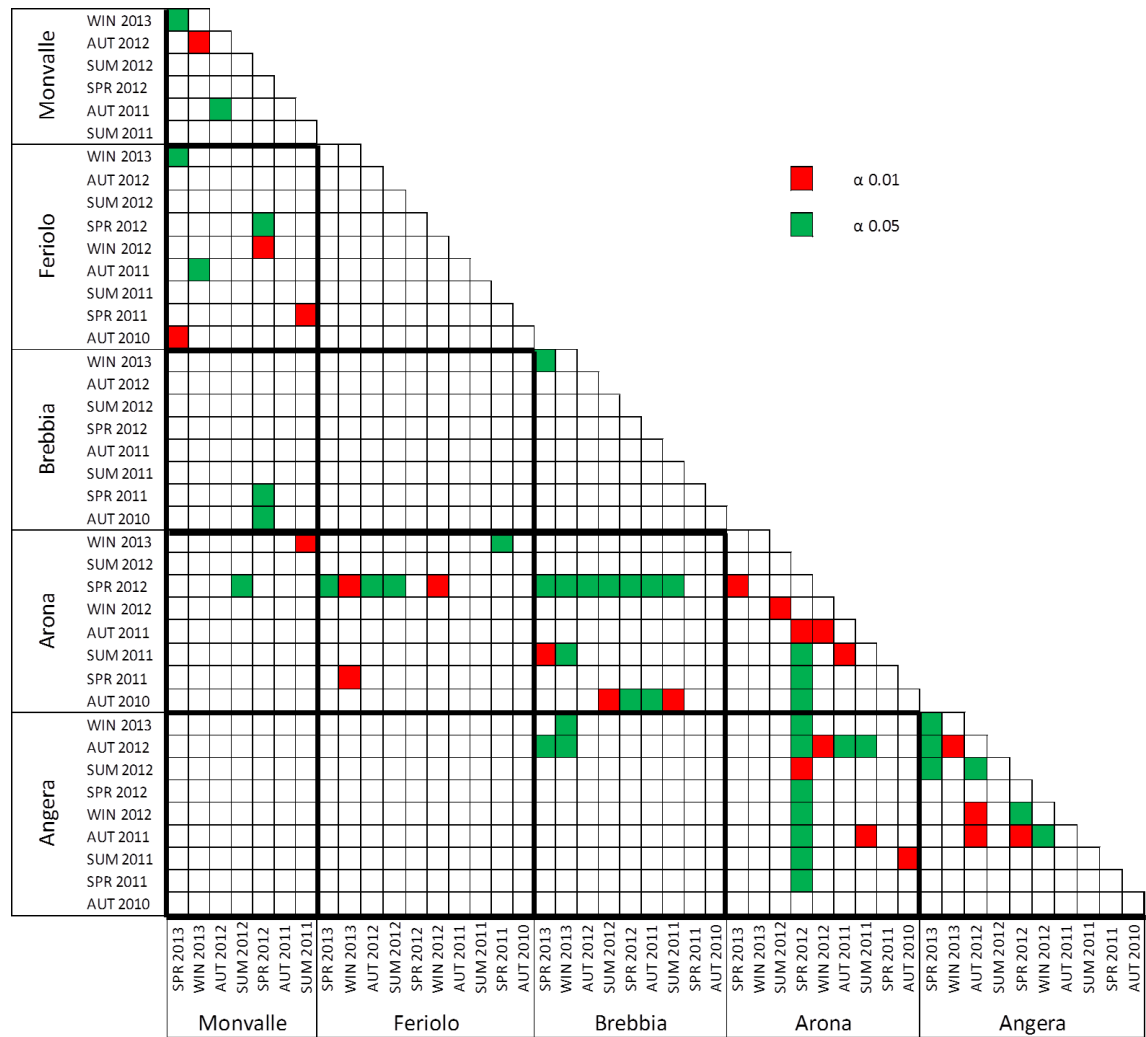


Figure 31: Distribution comparisons using Kolmogorov-Smirnov test
 Green: similarities between the two tested distribution are significant with $\alpha = 0.05$
 Red: similarities between the two tested distribution are significant with $\alpha = 0.01$

A comparison of size structure of population between alive organisms and empty shells are plotted (Figures from 32 to 36).

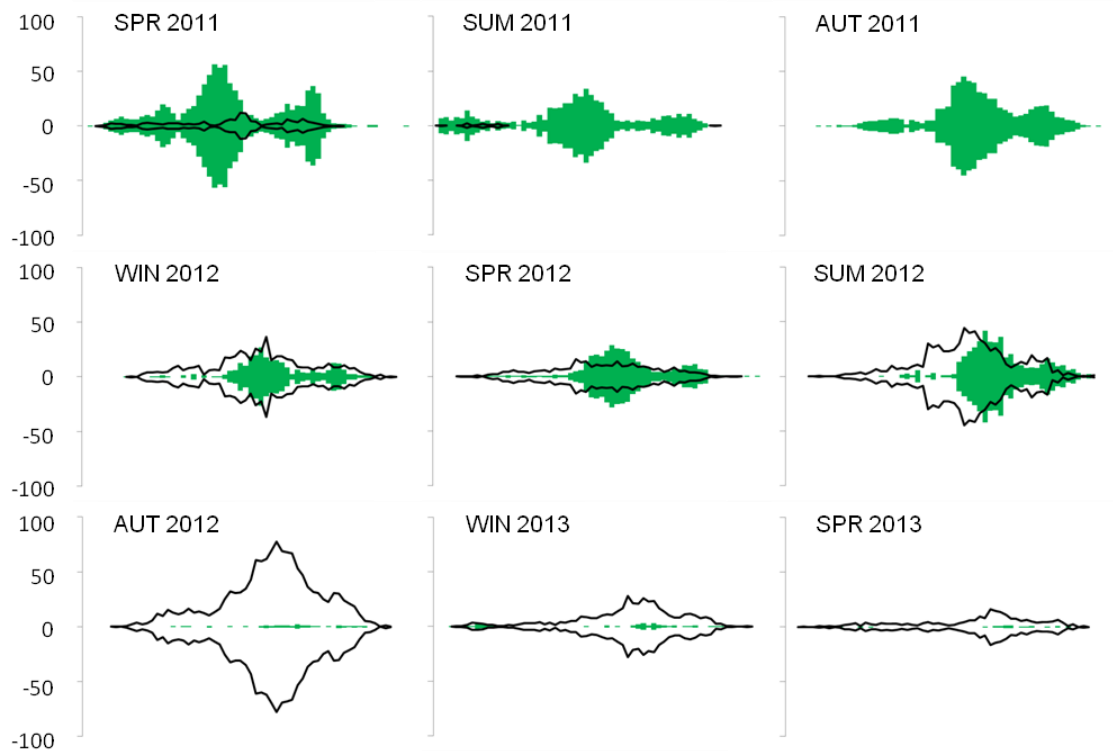


Figure 32: Comparison among alive specimens and empty shells collected at Angera
Data reported as densities (ind.m⁻²), size classes range = 0.5 mm

Until the Autumn of 2011, the population of Angera has high density and apparently a low mortality, after this period a decline in the mean density was observed, while there was an increase in the amount of empty shells (Figure 32). At the end of Summer 2012, due to a strong reduction of the water level (from 194.25 to 193.7 m) and with temperatures above 20°C, the density of *C. fluminea* dramatically decrease (about 20 times). This site has a particular shape that restricts the area colonisable by the clam. At the end of Summer 2012 only a few centimetres of water covering this area causing the mass mortality observed. The empty shells collected since Autumn 2012 persist for a relatively long period of time justifying the high densities recorded in the following sampling.

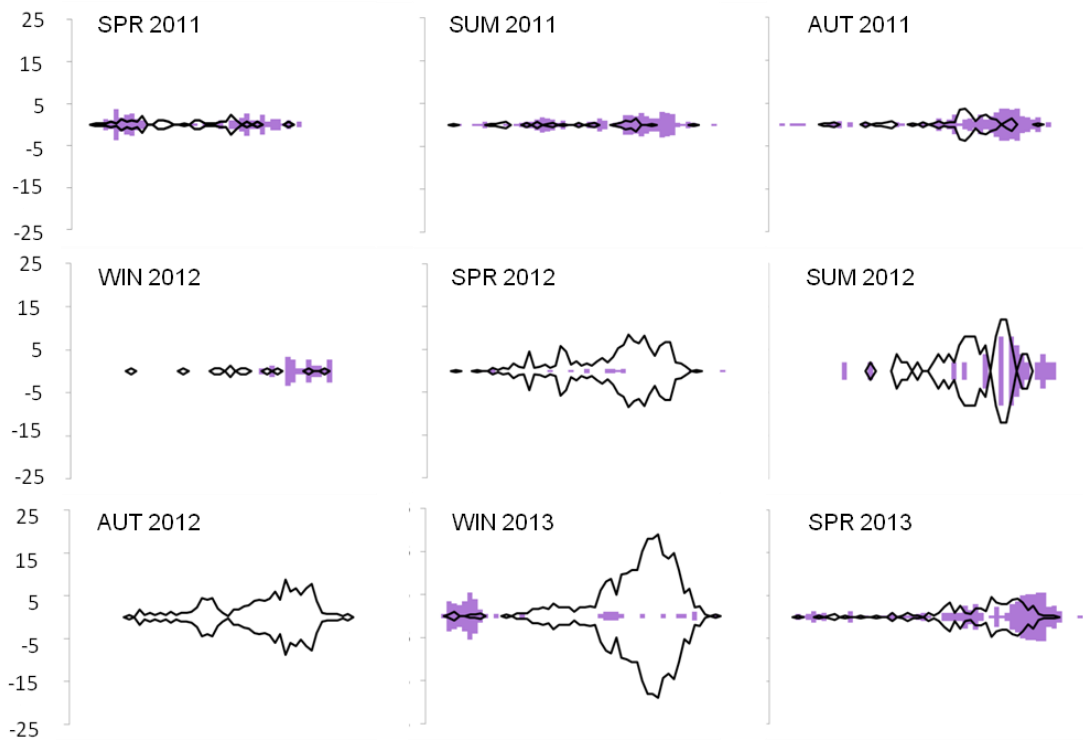


Figure 33: Comparison among alive specimens and empty shells collected at Arona
Data reported as densities (ind.m^{-2}), size classes range = 0.5 mm

Arona seems the site with the highest mortality rate, in fact often density of empty shells is comparable or higher than density of alive organisms (Figure 33).

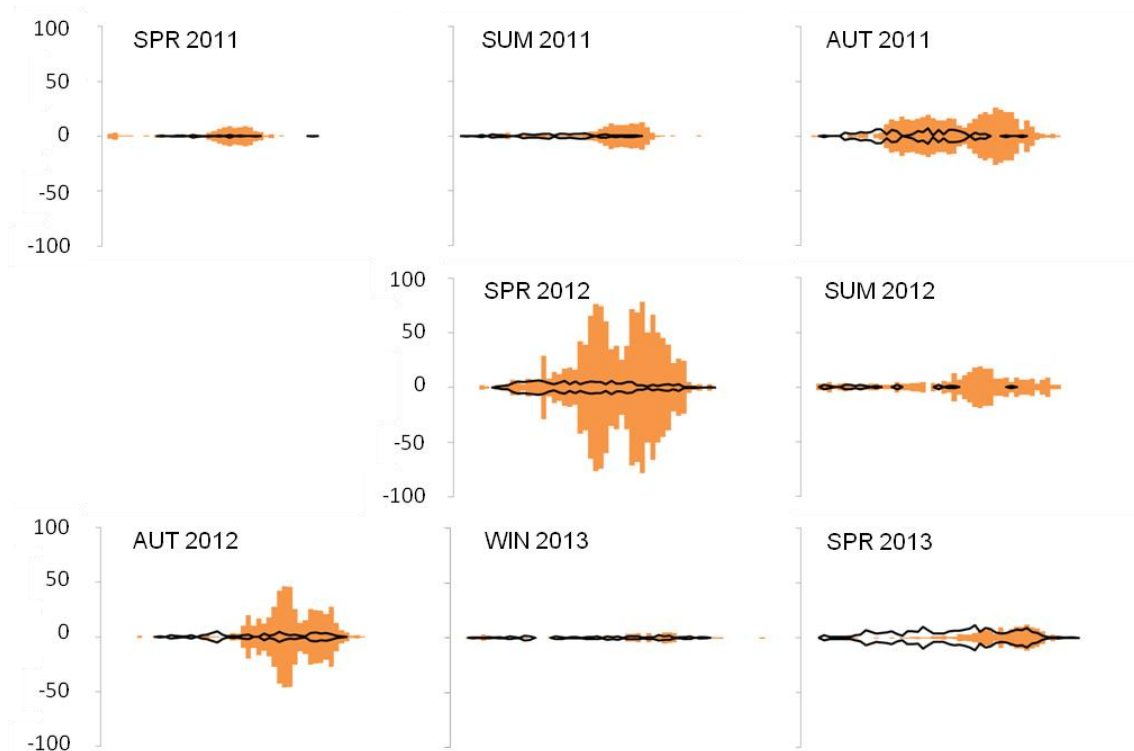


Figure 34: Comparison among alive specimens and empty shells collected at Brebbia
Data reported as densities (ind.m^{-2}), size classes range = 0.5 mm

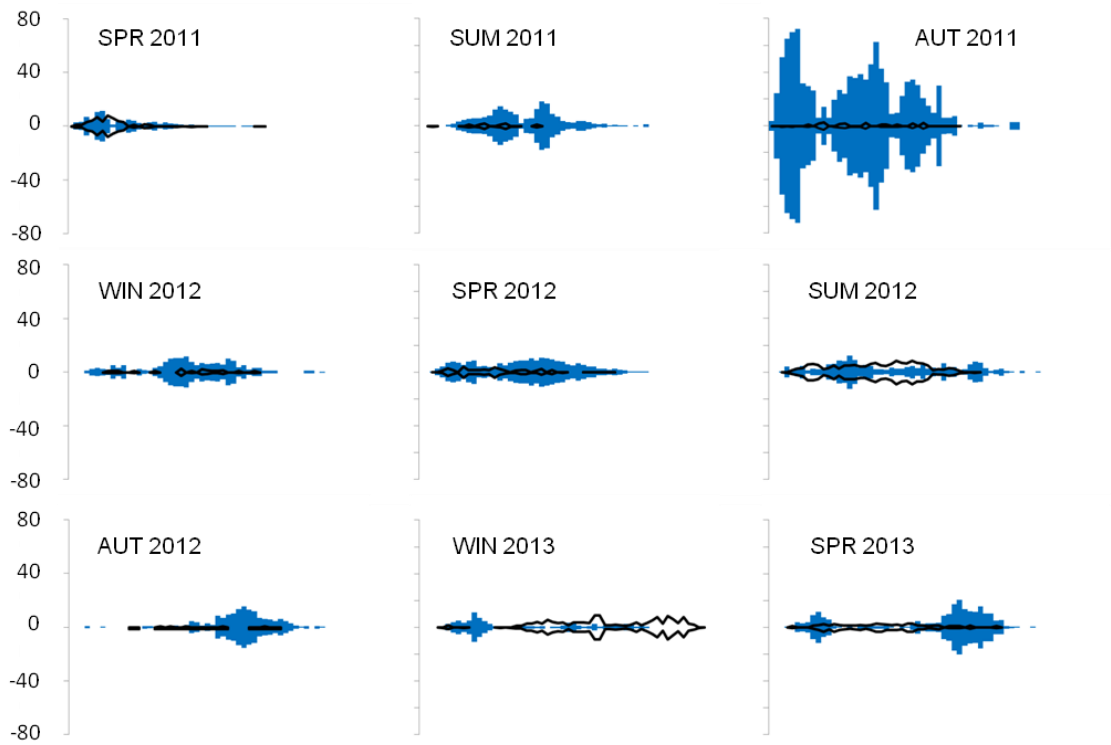


Figure 35: Comparison among alive specimens and empty shells collected at Feriolo
Data reported as densities (ind.m⁻²), size classes range = 0.5 mm

Brebbia and Feriolo present high densities of live animals, while empty shells are always less abundant than alive organisms, except in some cases (Winter 2013 and Spring 2013 at Brebbia, Spring 2011 and Summer 2012 in Feriolo; Figure 34 and Figure 35).

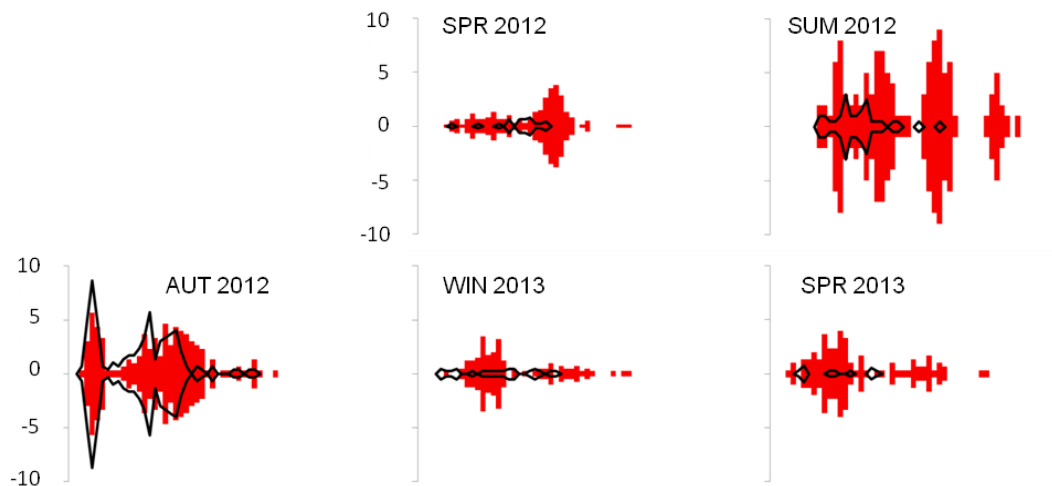


Figure 36: Comparison among alive specimens and empty shells collected at Monvalle
Data reported as densities (ind.m⁻²), size classes range = 0.5 mm

Monvalle is characterized to have a population constitutes mainly of small size and empty shells are recorded for the first time just after 9 month of observations (Summer 2011-Spring 2012; Figure 36). During this lapse of time this population almost duplicate the density.

As observed for Brebbia and Feriolo populations the presence of empty shells is lower than alive organisms, except in the Autumn of 2012, in this sampling the densities are rather similar.

In figure 37 is reported the comparison between the empty shells and live organisms collected in the same sample, while figure 38 illustrate the comparison between the empty shells and the animals collected in the following season.

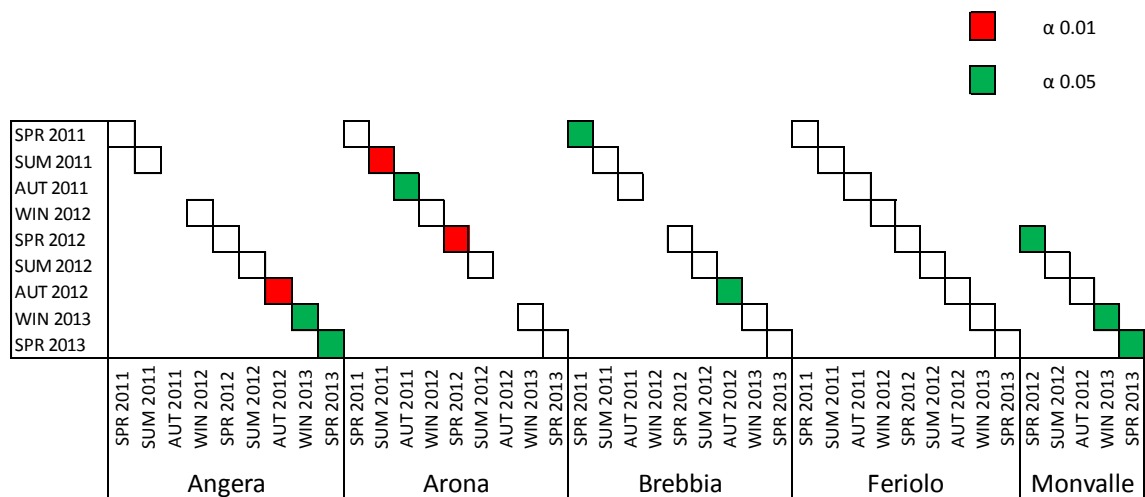


Figure 37: Distribution comparisons between empty shells and alive organisms of *C. fluminea* collected at the same time using Kolmogorov-Smirnov test
 Green: similarities between the two tested distribution are significant with $\alpha = 0.05$
 Red: similarities between the two tested distribution are significant with $\alpha = 0.01$

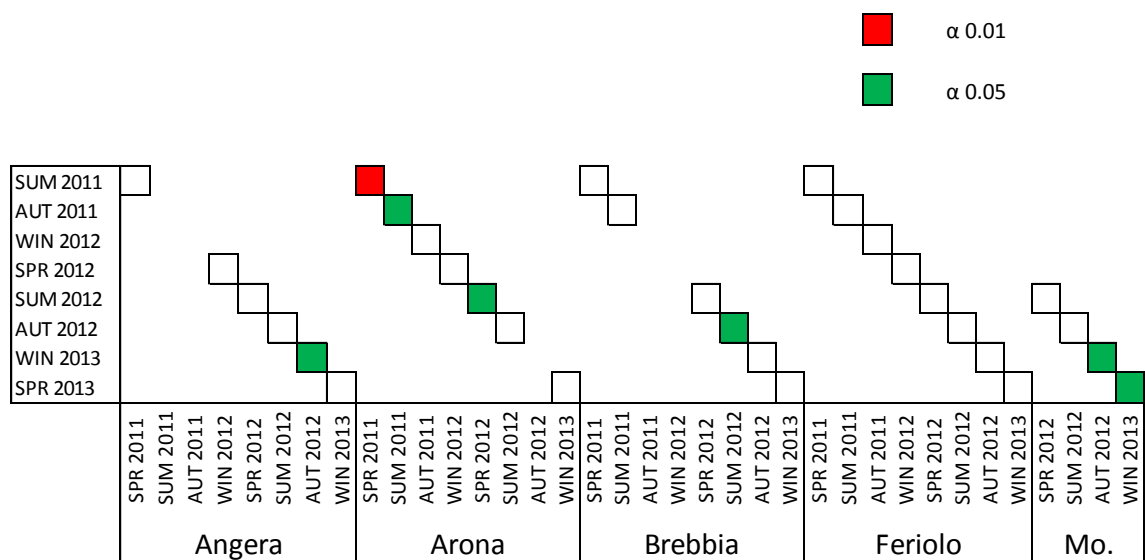


Figure 38: Distribution comparisons between empty shells and alive organisms collected during previous seasonal sampling of *C. fluminea* using Kolmogorov-Smirnov test
 Green: similarities between the two tested distribution are significant with $\alpha = 0.05$
 Red: similarities between the two tested distribution are significant with $\alpha = 0.01$

The results of the Kolmogorov-Smirnov and the figures from 32 to 36 suggest that mortality afflicts all size classes; there are no indications of age-specific mortality events. In addition, it's possible to hypothesize that the shells remain in the environment for a

rather long period. In fact, the presence of the shells is reduced in time, with a similar distribution found in the following samplings ($\alpha 0.05$).

3.4 Growth rate

3.4.1 Cage growth evaluation

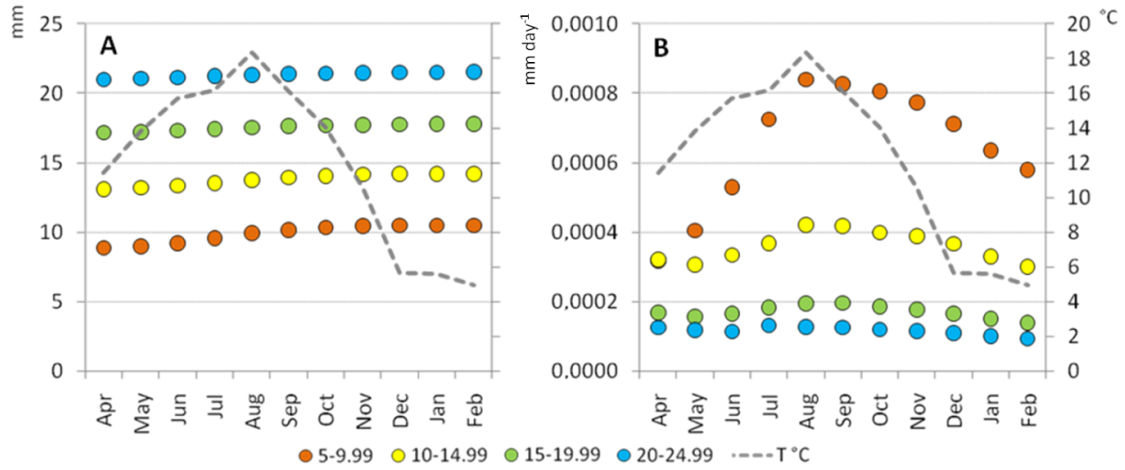


Figure 39: Angera growth in cages

A – Length of tested size classes (range 5 mm)

B – Growth rate of caged organisms distributed in size classes (range 5 mm)

With the passing of the seasons the lengths of the organisms of Angera population (Figure 39-A) increase for each size class. The mean growth rate (Figure 39-B) is higher for small size organisms, while growth slows with increasing of size and therefore age. The growth rate increases with higher temperature, reaching the maximum value in August. Subsequently the temperature decreases, implying a reduction of growth rate.

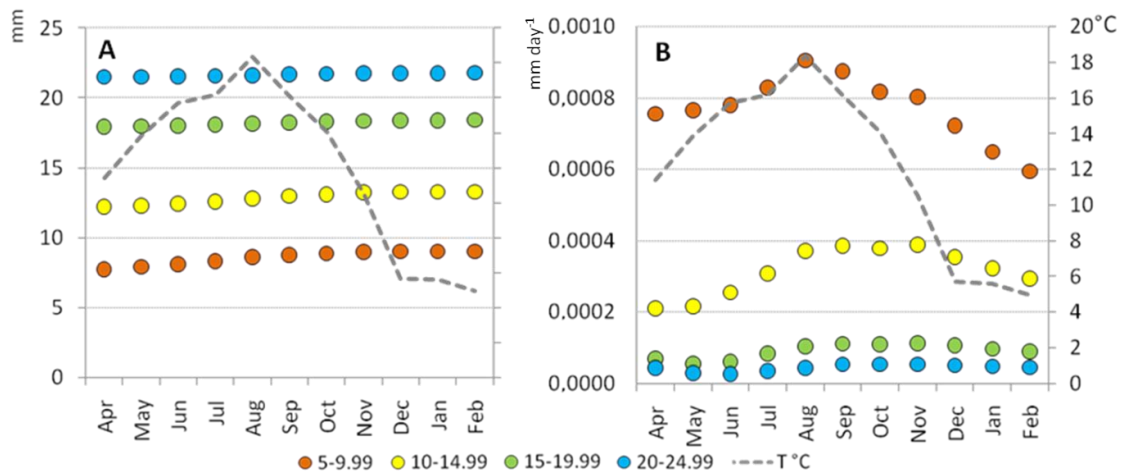


Figure 40: Brebbia growth in cages

A – Length of tested size classes (range 5 mm)

B – Growth rate of caged organisms distributed in size classes (range 5 mm)

Same observation emerge from results of Brebbia population (Figure 40 A and B). The increase in length is more pronounced for small size organisms, while larger specimens

show a slower growth rate. Also in this case the response of the growth rate is related to temperature variations.

The equations that best approach the points on the graphs are straight line equations. Figure 41 shows the results of the regression and table 18 the relative straight lines equations and the R² values.

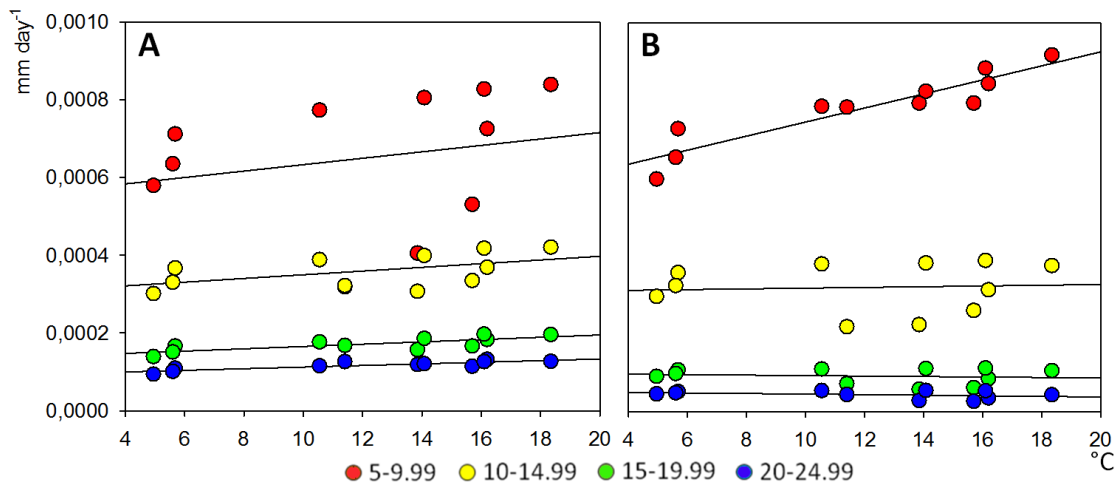


Figure 41: Linear regression of size classes growth rates
A – Angera B - Brescia

Considering larger organisms growth rates are less variable, while for the smaller size class the points that define the mean growth rate for a given temperature are more distant from the straight line. The Angera organisms with size class of 5 to 9.99 mm show the most variability, mainly due to the lack of animals of this size, more abundant in Brescia.

Table 18: Equations of linear regressions (Figure 41)

Size class	Angera		Brescia	
5-9.99	$y = 8 \cdot 10^{-6}x + 0.0006$	$R^2 = 0.0513$	$y = 2 \cdot 10^{-5}x + 0.0006$	$R^2 = 0.8529$
10-14.99	$y = 5 \cdot 10^{-6}x + 0.0003$	$R^2 = 0.2772$	$y = 9 \cdot 10^{-7}x + 0.0003$	$R^2 = 0.0049$
15-19.99	$y = 3 \cdot 10^{-6}x + 0.0001$	$R^2 = 0.599$	$y = -6 \cdot 10^{-7}x + 0.0004$	$R^2 = 0.0213$
20-24.99	$y = 2 \cdot 10^{-6}x + 9 \cdot 10^{-5}$	$R^2 = 0.7195$	$y = -7 \cdot 10^{-7}x + 5 \cdot 10^{-5}$	$R^2 = 0.1252$

The growth rates for each size class are tested to assess similarities and differences between the results obtained by the sub-samples of Angera and Brescia. The figure 42 shows the growth rates values observed for the two sites, while in table 19 are reported the results of the Student's t test used to compare the growth rates of the two examined samples.

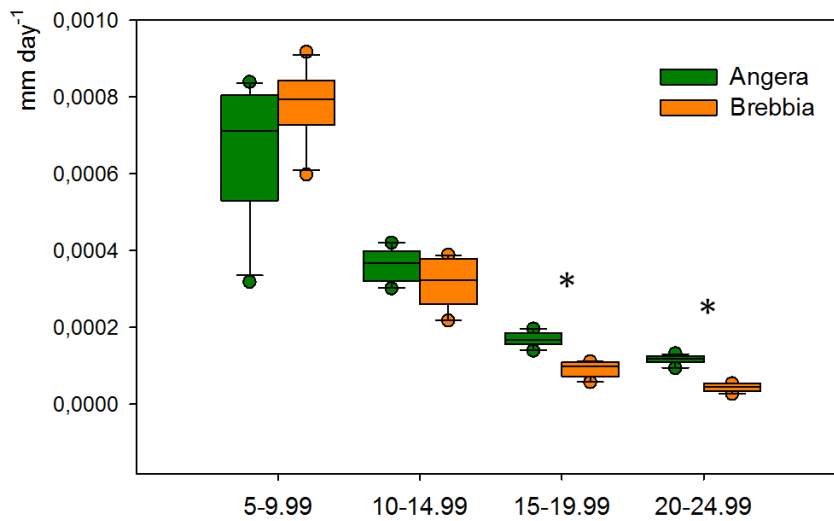


Figure 42: Size classes growth rate comparison between Angera and Brebbia

Despite individual variability, more marked in the smaller size classes (5-9.99 only for Angera and 10-15.99 mm for both), the Student's t test does not show a significant difference between the two populations. Significant differences in the growth rate are found for classes of larger size classes (15-19.99 and 20-24.99 mm). This difference can also be seen by observing figure 39, figure 40 and figure 42.

Table 19: t-test results of comparison between size classes growth rate of Angera and Brebbia populations ($\alpha = 0.05$)

Size class	T	DF	p	H ₀
5-9.99	-2.059	20	0.053	Accepted
10-14.99	1.804	20	0.086	Accepted
15-19.99	9.655	20	< 0.001	Rejected
20-24.99	15.754	20	< 0.001	Rejected

3.4.2 Field growth evaluation

Observing and comparing different size class structure of sampled populations is possible to trace cohorts growth, with the help of methodologies to identify in the most correct way possible the peaks of the distributions (Walford method (1946) and probability paper proposed by Harding (1949); Appendix 1-10). In fact, also looking figure 30 is possible to follow movement of cohorts over time. In following figures (from 43 to 47), every new cohort admissions is visualised in the lower part of the graphs. During time the picks that identify every cohort become more large, less high and more distant from origin of the y-axis. Defining every pick it is possible to evaluate the growth rate of the different cohorts. The cohorts peaks are interpolated by curves and it is possible to assume that these curves are segments of sigmoidal curves. This kind of curve is due to different growth rate of small and large individuals. Small organisms show a high growth (exponential curve), with increasing in size/age is associated a reduction of growth rate (asymptotic curve, tending to a maximum value of length).

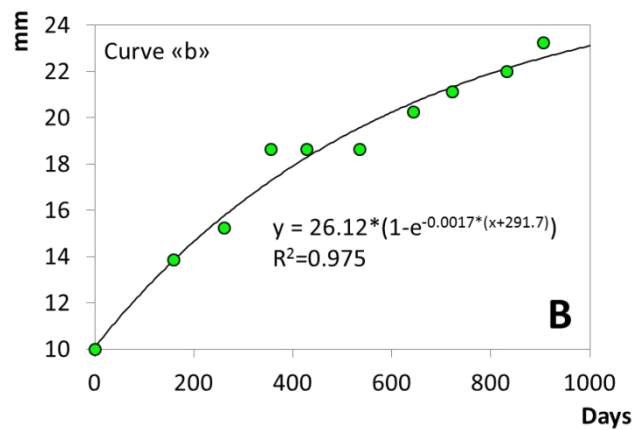
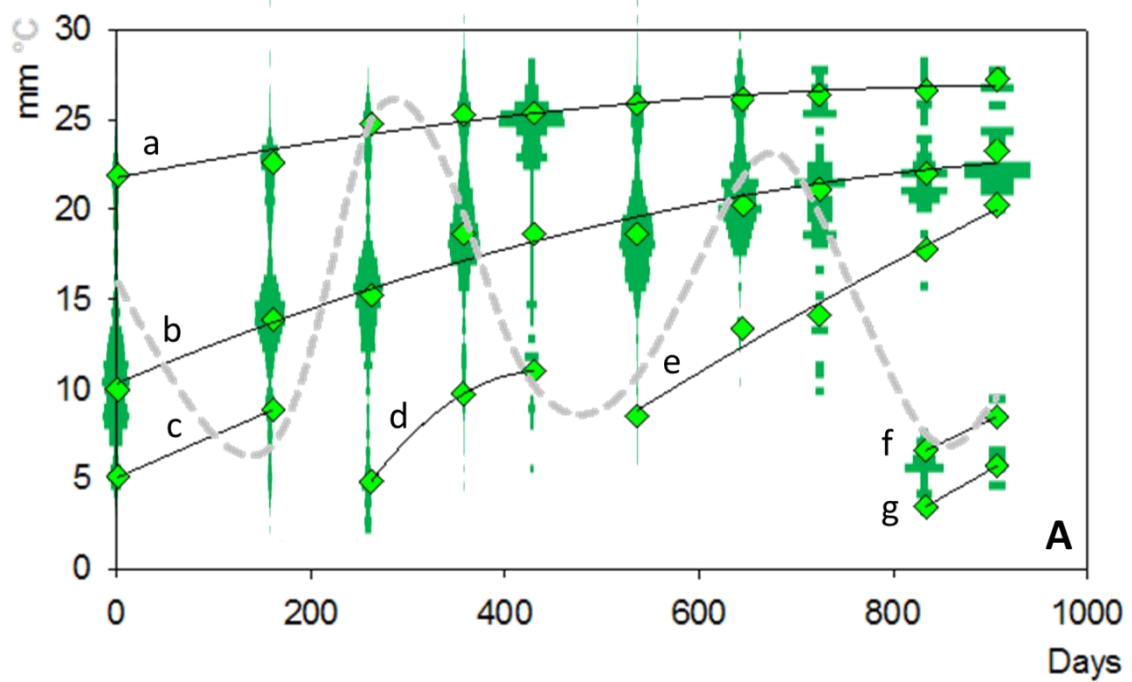


Figure 43: Population size structure and cohorts growth - Angera
 A – Curves defining cohorts growth
 B – von Bertalanffy growth curve

Table 20: Angera field growth rate curves equations (Figure 43)

Curve	Angera
Straight line	
a	$y=21.77+0.011x-5.56 \cdot 10^{-6}x^2$ $R^2=0.9534$
b	$y=10.34+0.023x-1.02 \cdot 10^{-5}x^2$ $R^2=0.9702$
c	$y=5.102+0.024x$ $R^2=1$
d	$y=-27.61+0.177x-2 \cdot 10^{-4}x^2$ $R^2=1$
e	$y=-11.72+0.043x-9.067 \cdot 10^{-6}x^2$ $R^2=0.9789$
f	$y=-14.79+0.026x$ $R^2=1$
g	$y=-22.21+0.031x$ $R^2=1$

Angera at the first sampling presents three cohorts: one close to 5 mm, the second around 10 mm and the last about 22 mm (Figure 43-A). At the following sampling this three cohorts are maintained and the organisms that constitute every cohort show an increase of length. During time the second and the third cohorts observed at the beginning are easily during the following sampling, while smaller size classes cohorts appear to be interrupted. Four different recruit events are evident (curves “c”, “d”, “e” and “g”). As highlighted from the curve equations (Table 20), relation between growth and time tends to an asymptote (maximum potential length), the von Bertalanffy formula fits with curve “b” with a k value of 0.0017 (Figure 43-B).

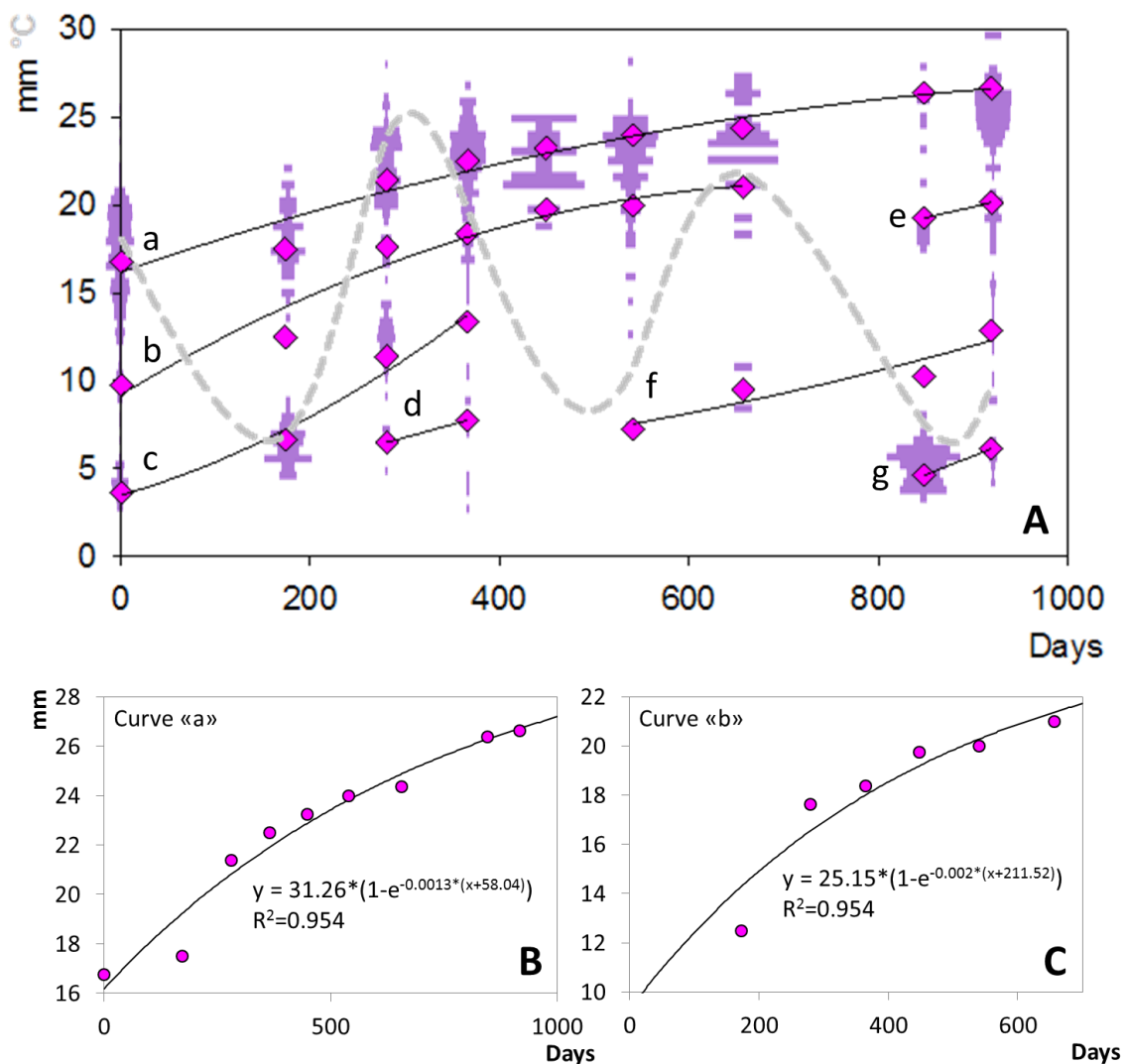


Figure 44: Population size structure and cohorts growth - Arona
 A – Curves defining cohorts growth
 B – von Bertalanffy growth curve

Table 21: Arona field growth rate curves equations (Figure 44)

Curve	Arona	
Straight line		
a	$y=16.19+0.019x-7.8 \cdot 10^{-6}x^2$	$R^2=0.9561$
b	$y=9.2+0.033x-2.21 \cdot 10^{-5}x^2$	$R^2=0.9581$
c	$y=3.49+0.016x+3.4 \cdot 10^{-5}x^2$	$R^2=0.9798$
d	$y=2.37+0.015x$	$R^2=1$
e	$y=8.8+0.012x$	$R^2=1$
f	$y=4.38+0.002x+7.23 \cdot 10^{-6}x^2$	$R^2=0.8805$
g	$y=-13.29+0.021x$	$R^2=1$

Arona presents a similar situation, with three cohorts (about 4 mm, 10 mm, 17 mm) during first sampling (Figure 44-A). In this case, due the scarcity of organisms usually collected, growth of cohorts appears not always clear. During Winter 2012 (fifth sampling, 448 days after the first one) just few animals were found. New recruitments are represented by curves “f” and “g”. In table 21 are reported equation of figure 44 curves with the relative R^2 values. The von Bertalanffy formula fits with curves “a” and “b” with k values of 0.0013 and 0.002 respectively (Figure 44-B).

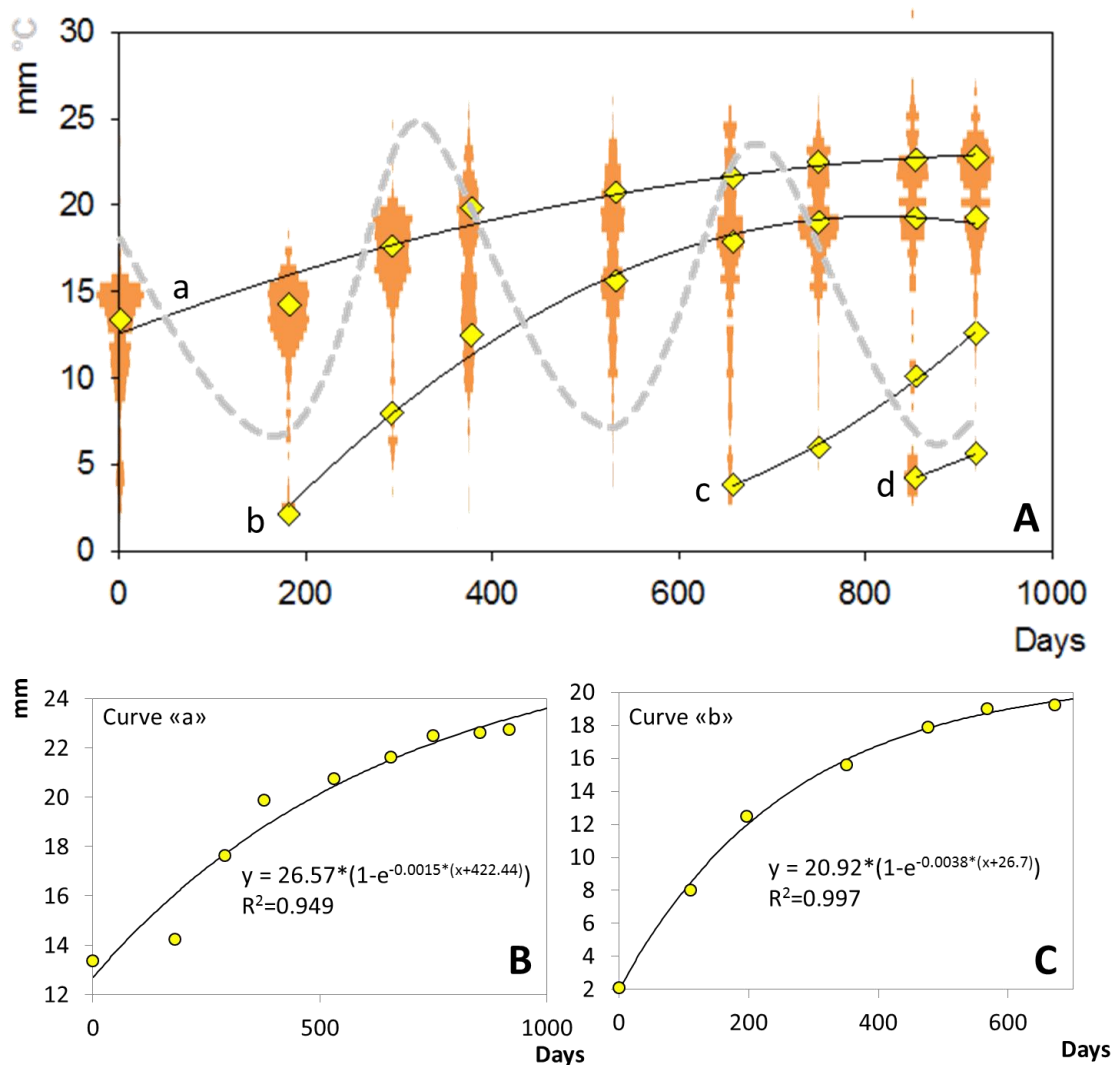


Figure 45: Population size structure and cohorts growth - Brebbia
 A – Curves defining cohorts growth
 B – von Bertalanffy growth curve

Table 22: Brebbia field growth rate curves equations (Figure 45)			
Curve	Brebbia		
Straight line			
a	$y=12.59+0.02x-9.97 \cdot 10^{-6}x^2$	$R^2=0.9552$	
b	$y=-8.35+0.068x-4.157 \cdot 10^{-5}x^2$	$R^2=0.9927$	
c	$y=10.89-0.043x+4.89 \cdot 10^{-5}x^2$	$R^2=0.9976$	
d	$y=-13.82+0.021x$	$R^2=1$	

Brebbia population shows a less complex situation during first sampling, with a big and more dispersed cohort around 13 mm and a new recruited one about 4 mm. This second cohort is composed by few animals and in following sampling is not easy to be detected. Curves are illustrated in figure 45-A and relative equations and R^2 values are reported in table 22. The new recruitments are observed in Spring 2011 (day 181, second sampling) and another event is recorded during Winter 2013 (day 853, eighth sampling). For curves

“a” and “b” is possible to apply von Bertalanffy formula, that return with k values of 0.0015 and 0.0038 respectively (Figure 45-B)

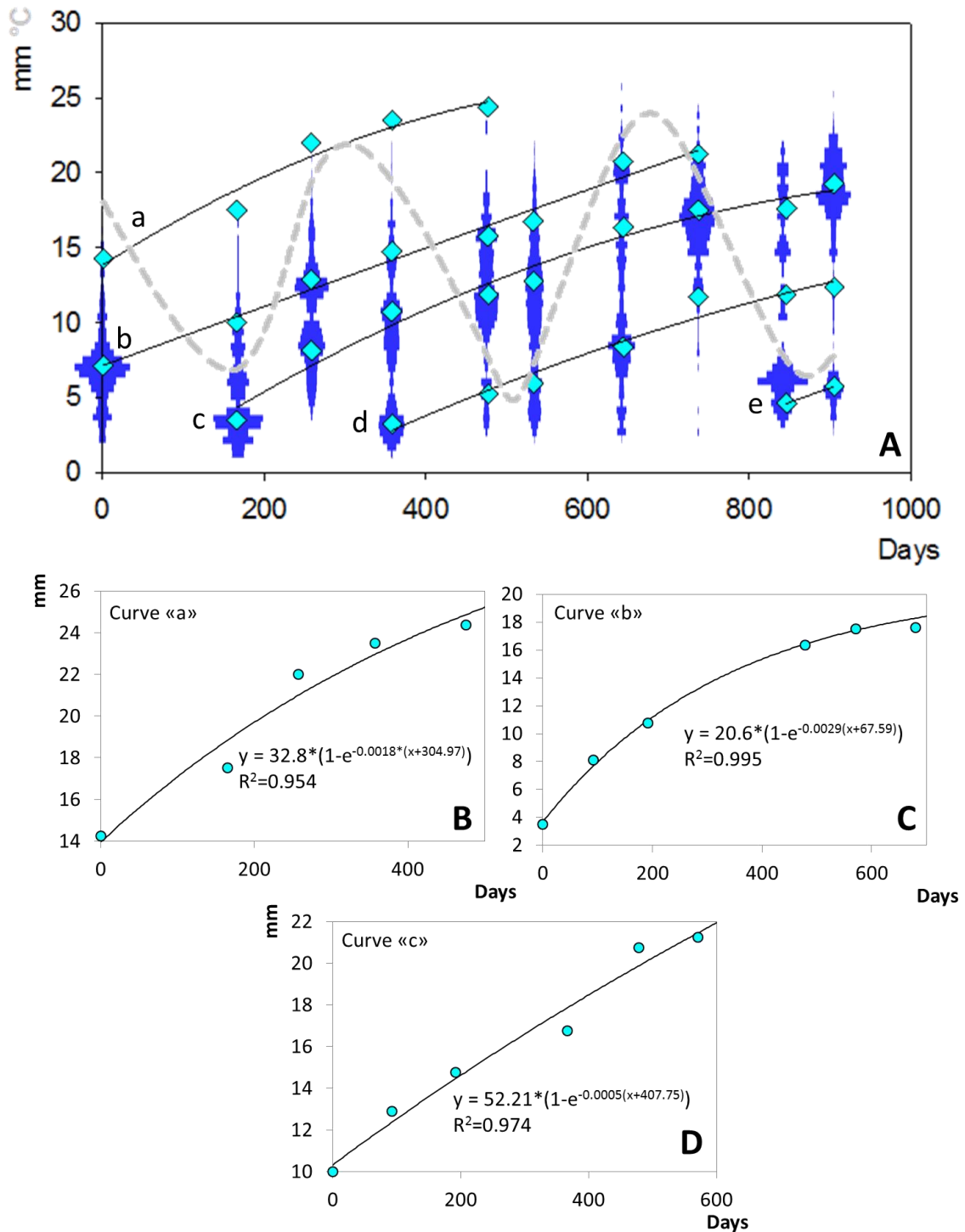


Figure 46: Population size structure and cohorts growth - Feriolo
A – Curves defining cohorts growth
B – von Bertalanffy growth curve

Table 23: Feriolo field growth rate curves equations (Figure 46)

Curve	Feriolo	
Straight line		
A	$y=13.85+0.034x-2.4 \cdot 10^{-5}x^2$	$R^2=0.9570$
B	$y=7.14+0.02x-4.44 \cdot 10^{-7}x^2$	$R^2=0.9803$
C	$y=-1.38+0.037x-1.64 \cdot 10^{-5}x^2$	$R^2=0.9747$
D	$y=-6.92+0.031x-1.002 \cdot 10^{-5}x^2$	$R^2=0,9637$
E	$y=-11.51+0.019x$	$R^2=1$

Feriolo, during first sampling, presents a population with two main cohorts, a dominant one about 7 mm and a smaller around 14 mm (Figure 46-A). Admissions of new cohorts are highlighted by curve “c” in Spring 2012 (165 day) and “d” in Autumn 2011 (day 357). Curves equations and R^2 values are reported in table 23; in this case von Bertalanffy formula fits with curves “a”, “b” and “c” (k values 0.0018, 0.0029 and 0.0005 respectively).

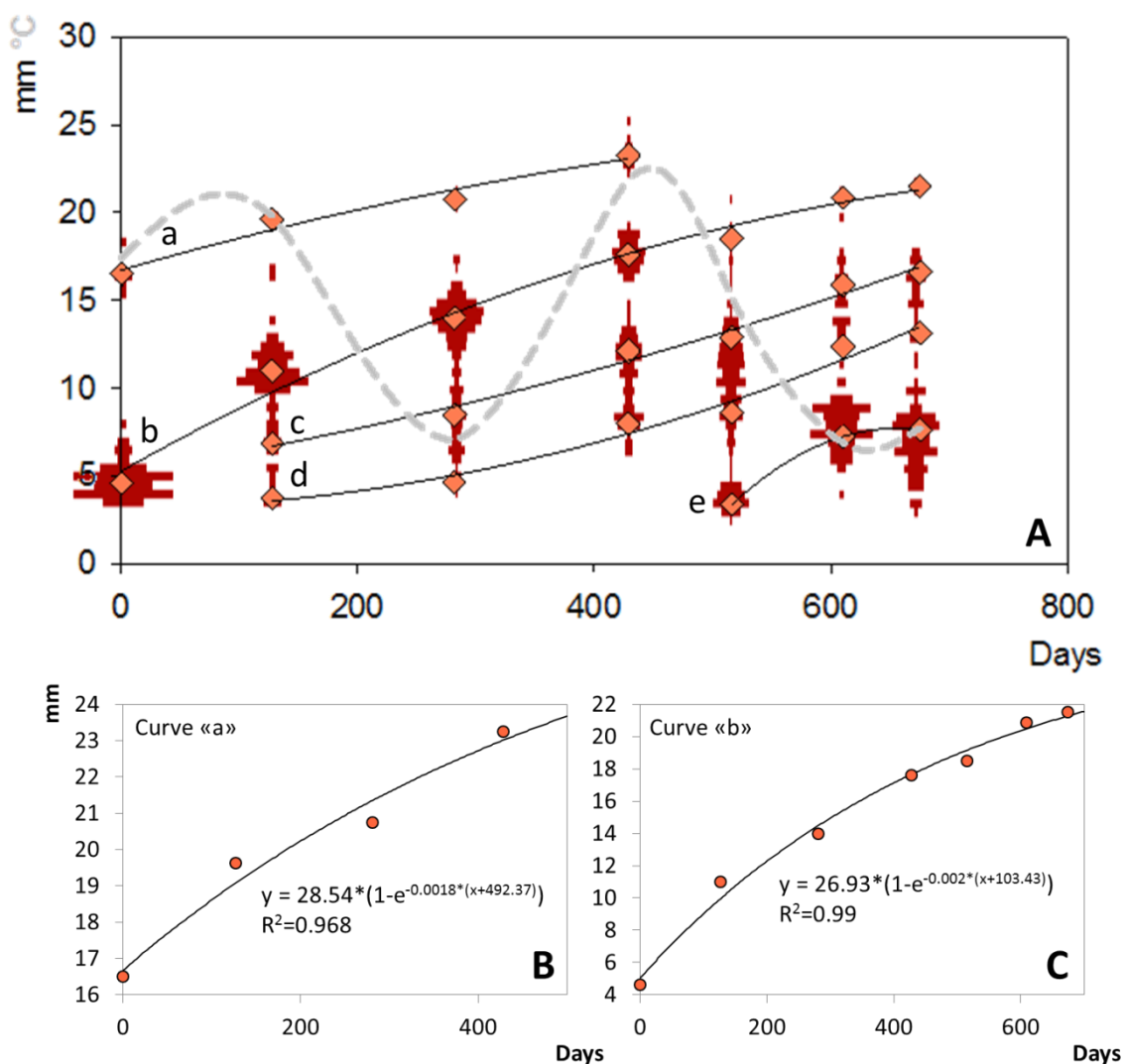


Figure 47: Population size structure and cohorts growth - Monvalle
 A – Curves defining cohorts growth
 B – von Bertalanffy growth curve

Table 24: Monvalle field growth rate curves equations (Figure 47)

Curve	Monvalle	
Straight line		
a	$y=16.72+0.019x-1.04 \cdot 10^{-5}x^2$	$R^2=0.9663$
b	$y=5.25+0.038x-2.11 \cdot 10^{-5}x^2$	$R^2=0.9814$
C	$y=5.2+0.011x+1.01 \cdot 10^{-5}x^2$	$R^2=0.9858$
D	$y=3.19+4 \cdot 10^{-4}x+2.21 \cdot 10^{-5}x^2$	$R^2=0.9778$
E	$y=-88.08+0.292x-2 \cdot 10^{-4}x^2$	$R^2=1$

Monvalle, during Summer 2011 (first sampling in this site, day 0) presents two cohorts, one less represented around 16 mm and the dominant one with pick about 4.5 mm. The recruitment defined with curve “e” is evident. Curve equations and R^2 values are reported in table 24 and fit with von Bertalanffy formula in case of curves “a” and “b” (k values 0.0018 and 0.002 respectively).

The k value is related to the daily growth rate of analysed cohort for the whole period. Usually values are included between 0.001 and 0.002 mm day⁻¹, with a maximum reached in Brebbia (0.0038 mm day⁻¹) and a minimum in Feriolo (0.0005 mm day⁻¹). Growth rates are evaluated dividing sampled organisms in size classes (range width: 5 mm; Figure 48).

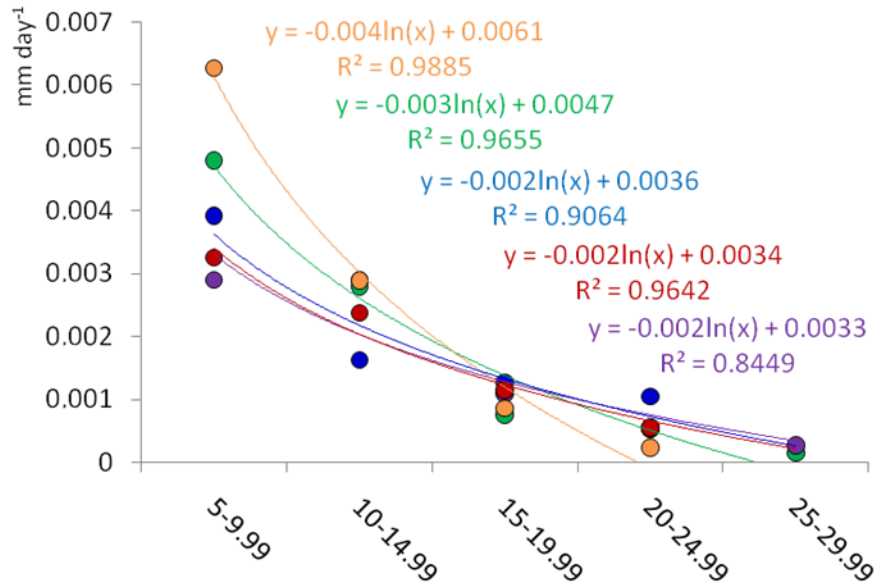


Figure 48: Field growth rate estimations over size classes
Curvilinear regression of daily growth rate (mm day⁻¹) over size classes (range=5 mm)

Growth rate is higher for organisms with reduced length/age, while larger organisms show a slower growth. Observing figure 49 Arona, Feriolo and Monvalle curves appear more close, while Angera and Brebbia present higher values of growth rate for the smaller organisms. These differences are less pronounced for bigger organisms.

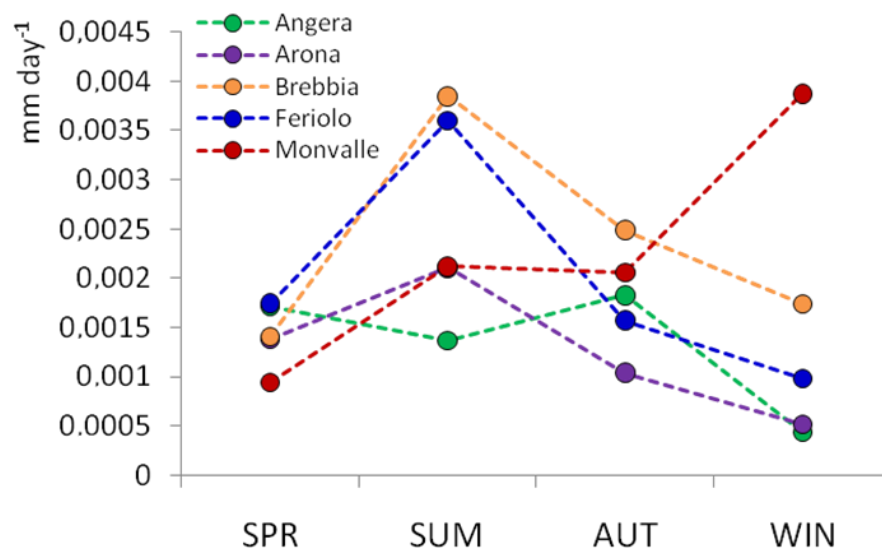


Figure 49: Seasonal field growth rates estimation

Using data from the growth rate in field it is possible to proceed with calculation of mean growth rate per season.

Figure 49 illustrates an increase of growth rate from Spring to Summer in every site, exception made for Angera. From Summer to Autumn the growth rate decreases at the sites of Arona, Brebbia and Feriolo; the trend is more pronounced in this last one. At Monvalle it is recorded a slight variation, while at Angera the growth rate increases. Upon arrival of the coldest months the growth rate undergoes a further decrease (Arona, Brebbia, Feriolo, higher decline observed at Angera).

The apparent decrease of winter growth observed at Monvalle seems to be due to shortage of sampling made in this season. In fact this site was sampled only once in Winter (2013) when a lot of small organisms, subject to high growth rate, were collected.

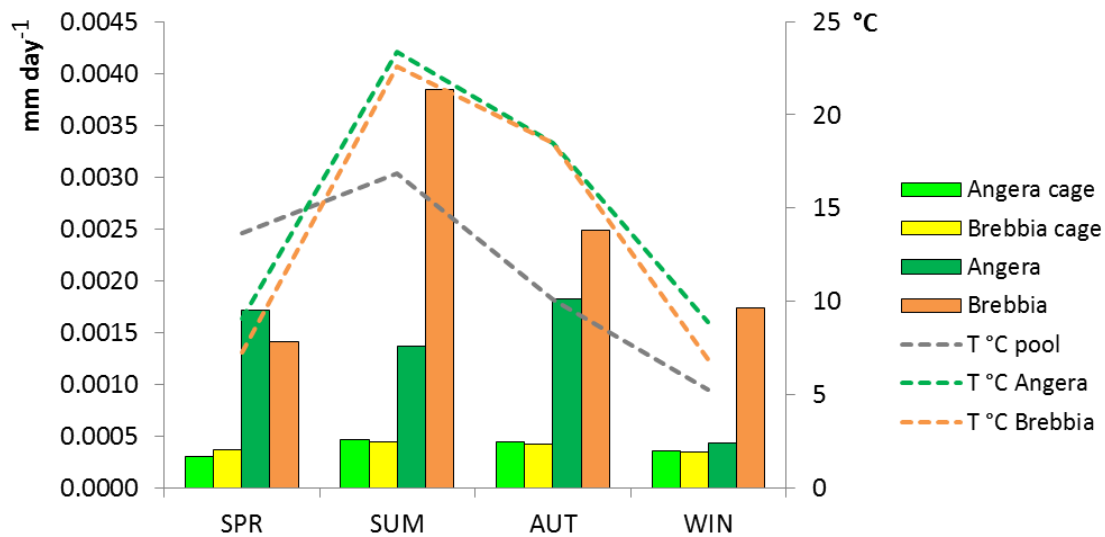


Figure 50: Cage and field growth rate comparison (Angera and Brebbia)

The figure 50 illustrates the comparison of cage growth rates (semi-natural conditions) with the field growth values estimated for the populations of sampling sites (Angera and Brebbia). The growth rate observed in semi-natural conditions is always lower than the field estimations.

In Spring, the field growth rate is from about 4 (Brebbia) to almost 6 times higher (Angera). In Winter, the growth observed in Angera is similar to that observed in the cages (1.2 times higher). In Summer and Autumn, greater differences are observed for the organisms of Brebbia (8 and 6 times higher than the cages), while Angera population presents values about 3 and 4 times higher comparing field and cages observations.

3.5 Reproduction

From the weekly observation of larval stages contained in the internal demibranchs it is possible to investigate the reproductive period and larval stage development of *C. fluminea* in this environment.

Data collected from cages kept in semi-natural conditions are compared with those of the site of Feriolo (Figure 51).

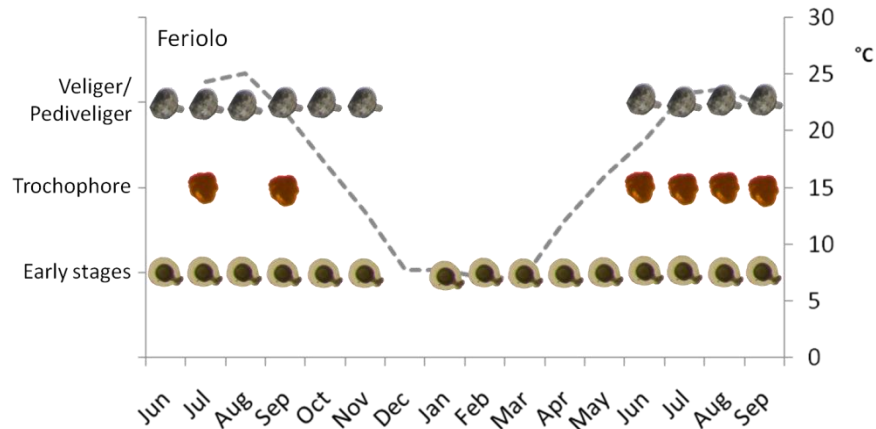


Figure 51: Larvae stages observed at Feriolo

C. fluminea in Lake Maggiore reproduces once a year (Figure 51). The production of new individuals occurs without interruption throughout the warm period of the year (from late Spring until Autumn).

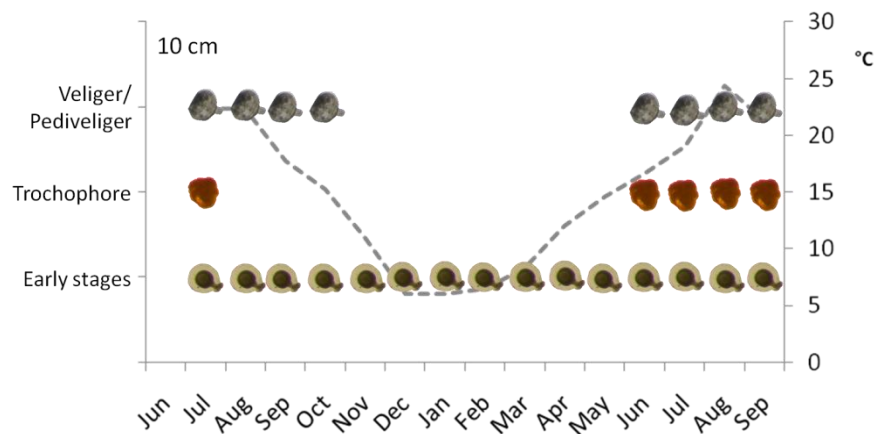


Figure 52: Larvae stages observed at 10 cm depth

For animals kept at 10 cm depth the production of Veliger/Pediveliger stops from November to June (Figure 52). Compared to organisms collected at Feriolo this interruption lasts a month longer.

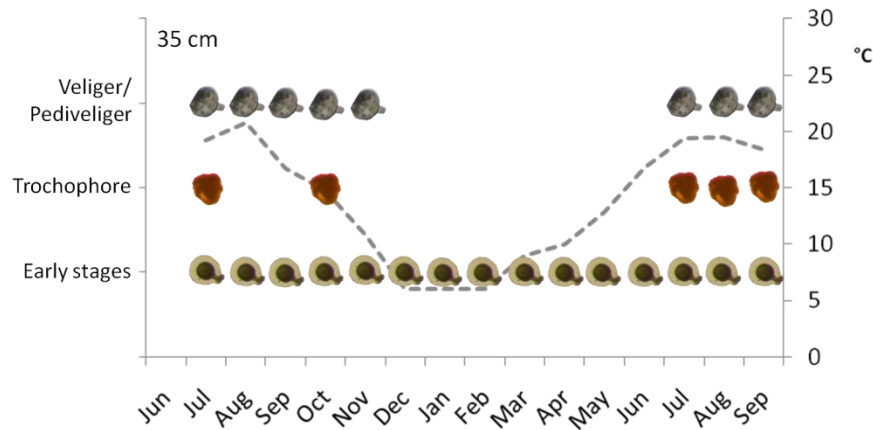


Figure 53: Larvae stages observed at 35 cm depth

At a depth of 35 cm a further reduction of the breeding period is observed, with a restart of Veliger/Pediveliger production from July (Figure 53).

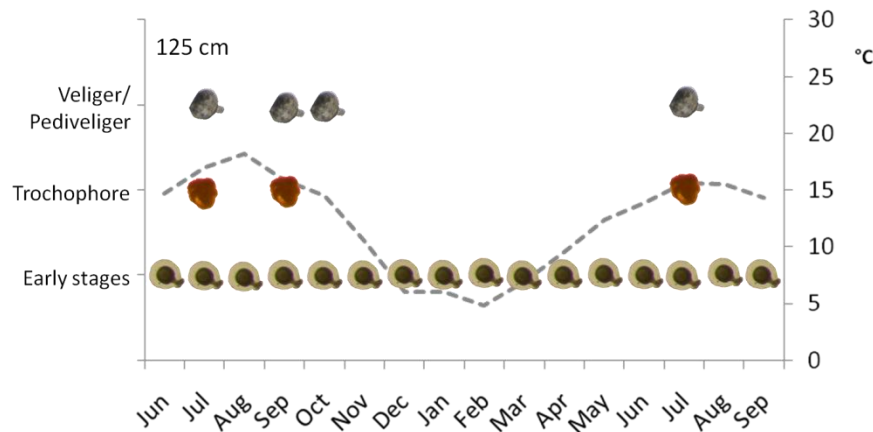


Figure 54: Larvae stages observed at 125 cm depth

At depth of 125 cm, the production of Veliger/Pediveliger is further reduced (Figure 54). This stage is found only in the months of July, September and October. In all conditions tested, the production of the early stages is observed throughout the year. This suggests that *C. fluminea* constantly invests part of its metabolic energy in reproduction. The maturation of the larval stages takes place at a temperature not below 15 °C.

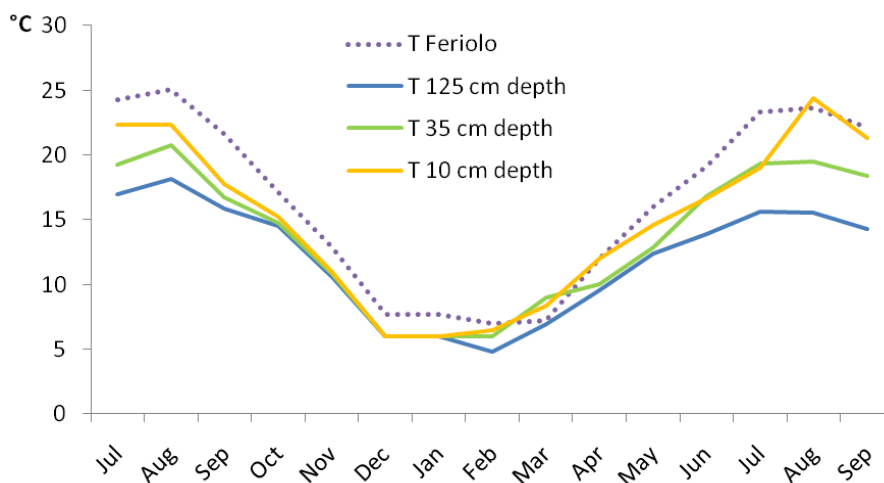


Figure 55: Comparison among temperatures recorded in tested conditions

The differences observed can be ascribed, at least in part, to different temperatures to which organisms are subject. In fact, the temperatures recorded in Feriolo are always higher than the artificial pool (except March; Figure 55). In fact, the water in the pool is exchanged twice a day with water pumped from the lake about 20 meters depth. The temperature is warmer close to the surface (10 cm depth) and decrease with increasing depth (35 and 125 cm depth).

Veliger/Pediveliger production results prolonged during the time for those animals subject to increasing temperatures.

Table 25: Mean individual larval production (larvae ind.⁻¹)

Site	AUT 10	SUM 11	AUT 11	SUM 12	AUT 12
Angera	314±382	1250±1195	2380±1479	2163±1838	297±374
Arona			1183±1361		
Brebbia	264±153	635±849	713±853	1103±993	65±82
Feriolo	193±155	324±344		188±272	213±231
Monvalle			767±842		538±507

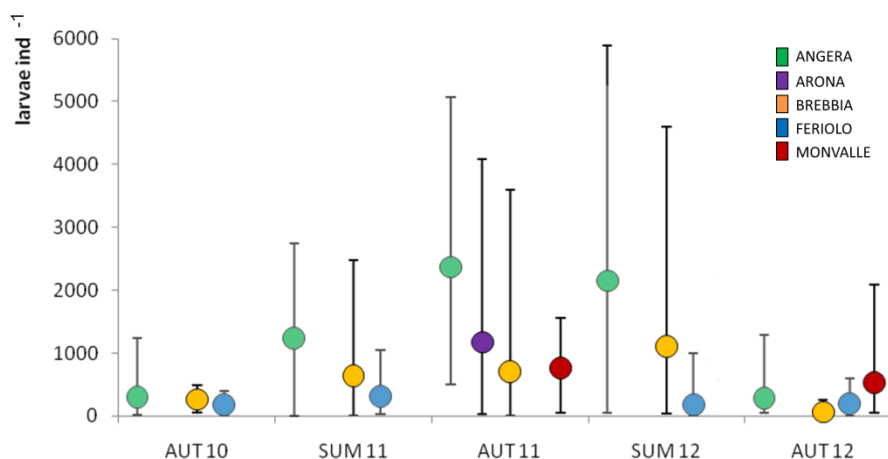


Figure 56: Individual larvae production in the five sampling sites

The individual larval production (Table 25 and Figure 56) shows some differences among organisms collected at the five sampling sites.

Angera, until Summer 2012, is the site presenting greater individual production of Veliger/Pediveliger (with a maximum of 5900 larvae ind.⁻¹). The highest mean value is observed in this site (2380 larvae ind.⁻¹ in Autumn 2011).

In order of succession for individual production, follow individuals collected in Brebbia. Until sampling of Summer 2012, in this site a production increase is observed (maximum value 4600 larvae ind.⁻¹ and mean 1103 larvae ind.⁻¹).

At Arona, given the scarcity of organisms that were collected during sampling, a single reproductive episode is observed (Autumn 2011: mean=1183 larvae ind.⁻¹, max=3904 larvae ind.⁻¹). The Veliger/Pediveliger production at Monvalle has been detected in two samplings: Autumn 2011 (mean 767 larvae ind.⁻¹ and maximum 1564 larvae ind.⁻¹) and Autumn 2012 (mean 538 larvae ind.⁻¹ and maximum 2104 larvae ind.⁻¹). At Feriolo are recorded the lowest values (mean 324 larvae ind.⁻¹ in Summer 2011 - 188 larvae ind.⁻¹ Summer 2012).

Table 26: Esteemed larval production per square meter at the five sampling sites (larvae m⁻²)

Site	AUT 10	SUM 11	AUT 11	SUM 12	AUT 12
Angera	14586	19420	579514	394550	2025
Arona			3904		
Brescia	15317	25062	164061	296935	7059
Ferriolo	496	5071		7832	7690
Monvalle			306		10774

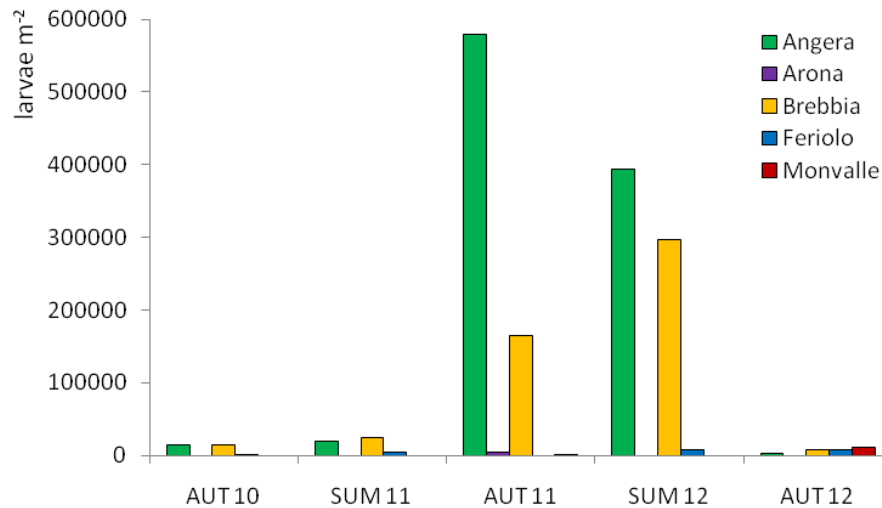


Figure 57: Larval production per square meter (larvae m⁻²) esteemed during sampling activities at the five sites

Calculating the percentage of reproductive individuals of different size classes on total population, it is possible to estimate the reproductive potential per square meter at the five sites (Table 26 and Figure 57).

In Autumn 2010, Angera and Brebbia populations present values higher than those of the other stations, almost 30 times the value of Ferriolo (496 larvae m⁻²). In Summer 2011 in Angera, Brebbia and Ferriolo the number of larvae produced per square meter increases (approximately 33, 64 and 922%, respectively).

The maximum values are reached at Angera (5,795,134 larvae m⁻² in Summer 2011) and Brebbia (296,935 larvae m⁻² in Autumn 2011). High values of these two sites are due to high population densities, to high percentage of reproductive organisms and to their abundant individual production.

Arona population, during Autumn 2011, produces a value of 3904 larvae m⁻². Monvalle moved between 306 in Autumn 2011 and 10774 the following year.

A detailed analysis of the number of larvae produced (per individual and per square meter; from figures 58 to 67, respectively A and B) divided by the percentage of reproductive by size classes has been performed.

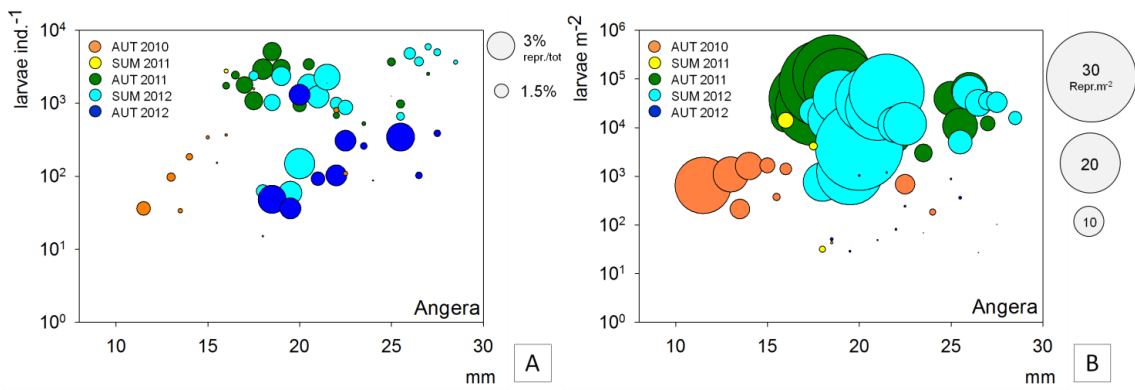


Figure 58: Larvae production over length (size classes 0.5 mm) at Angera. A = individual production, bubble size = reproductive organisms percentage of sampled population. B = larvae production per square meter, bubble size = reproductive organisms per square meter

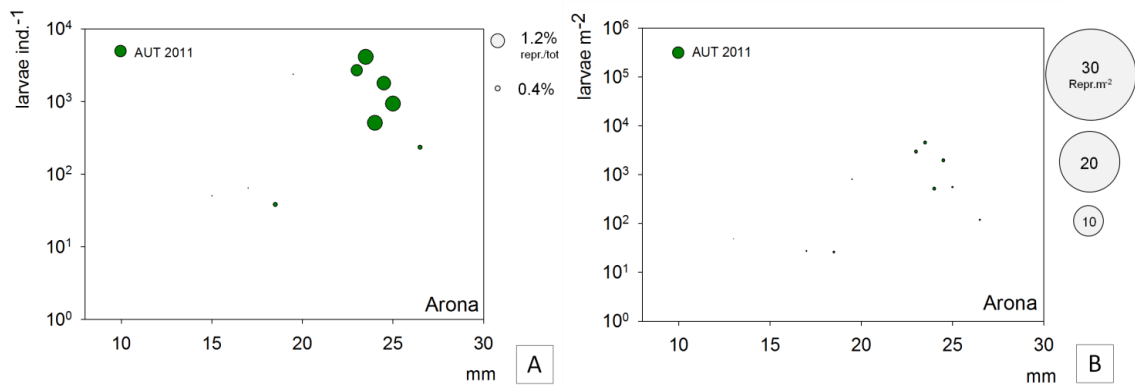


Figure 59: Larvae production over length (size classes 0.5 mm) at Arona. A = individual production, bubble size = reproductive organisms percentage of sampled population. B = larvae production per square meter, bubble size = reproductive organisms per square meter

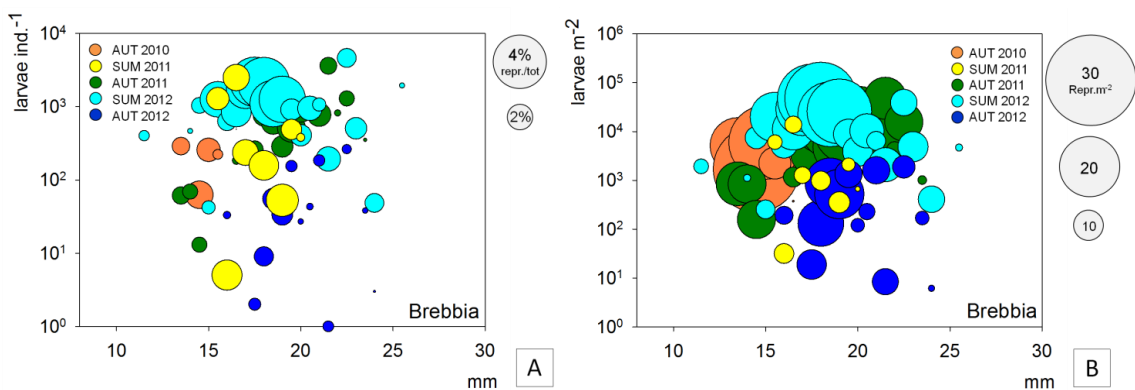


Figure 60: Larvae production over length (size classes 0.5 mm) at Brebbia. A = individual production, bubble size = reproductive organisms percentage of sampled population. B = larvae production per square meter, bubble size = reproductive organisms per square meter

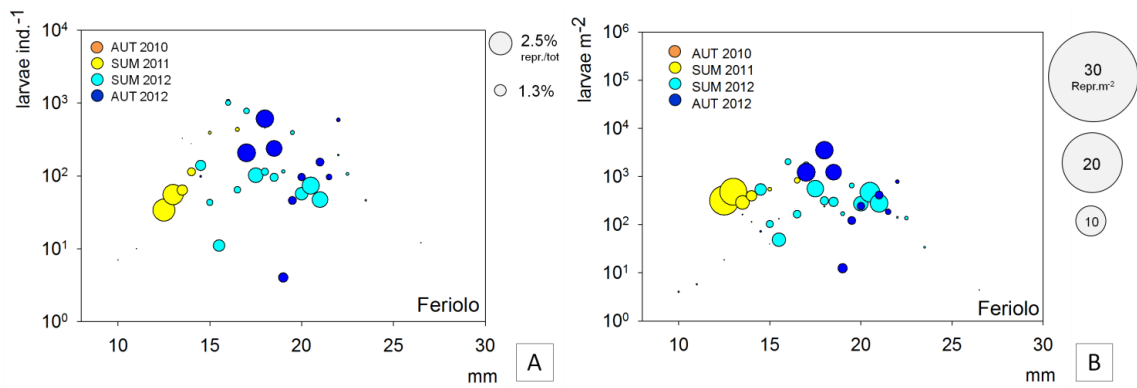


Figure 61: Larvae production over length (size classes 0.5 mm) at Feriolo. A = individual production, bubble size = reproductive organisms percentage of sampled population. B = larvae production per square meter, bubble size = reproductive organisms per square meter

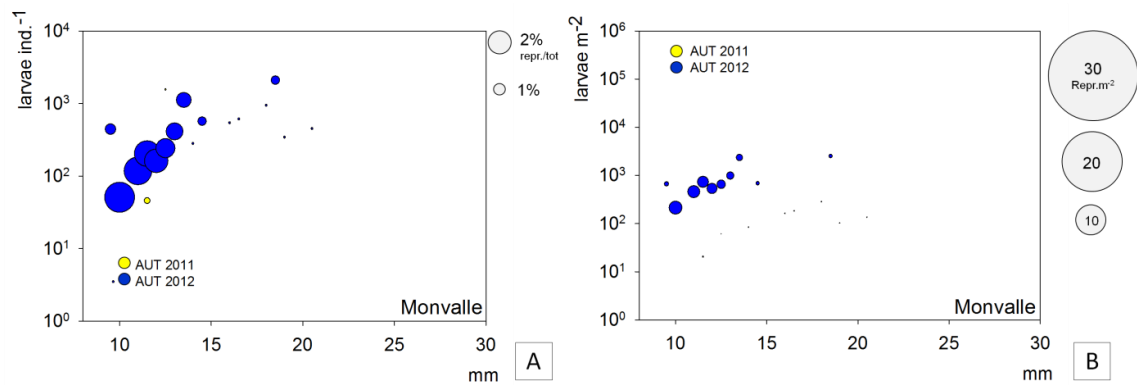


Figure 62: Larvae production over length (size classes 0.5 mm) at Monvalle. A = individual production, bubble size = reproductive organisms percentage of sampled population. B = larvae production per square meter, bubble size = reproductive organisms per square meter

During time, populations in the different sites present organism with bigger dimensions, due to growth of their components.

As consequence, it is observed a length increase of reproductive organisms. In fact, reproductive size classes of first sampling (Autumn 2010 in Angera and Feriolo, Autumn 2011 in Monvalle) are subject to variations during the following sampling: number of reproductors, size class and relative larval production is increasing (Figures from 58 to 62). Brebbia population is an exception: reproductors are distributed in a large scale of size classes (among 12 and 25 mm) that remains constant during time.

In the sites where larval production is recorded in Summer and Autumn, it is possible to observe that in 2011 larvae produced per square meter are more abundant in Autumn (exception made for Brebbia), while the year after larval production is higher in Summer.

At Arona, during the sole sampling where larval production is observed, reproductors are concentrated in a size class among 22 and 26 mm. Reprodutor percentage is rather low (less than 15% of sampled population), with a high individual production, mostly for organism of 23-24 mm length (mean of individual production about 23000 larvae).

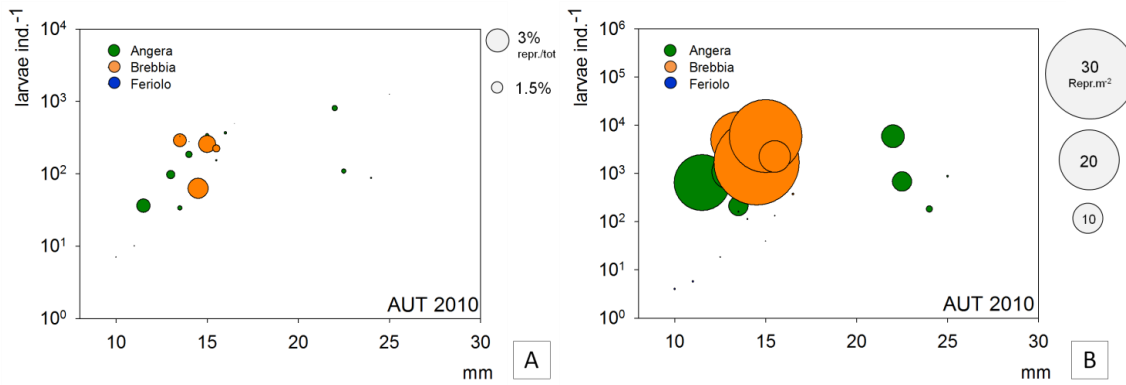


Figure 63: Larvae production over length (size classes 0.5 mm) in Autumn 2010. A = individual production, bubble size = reproductive organisms percentage of sampled populations. B = larvae production per square meter, bubble size = reproductive organisms per square meter

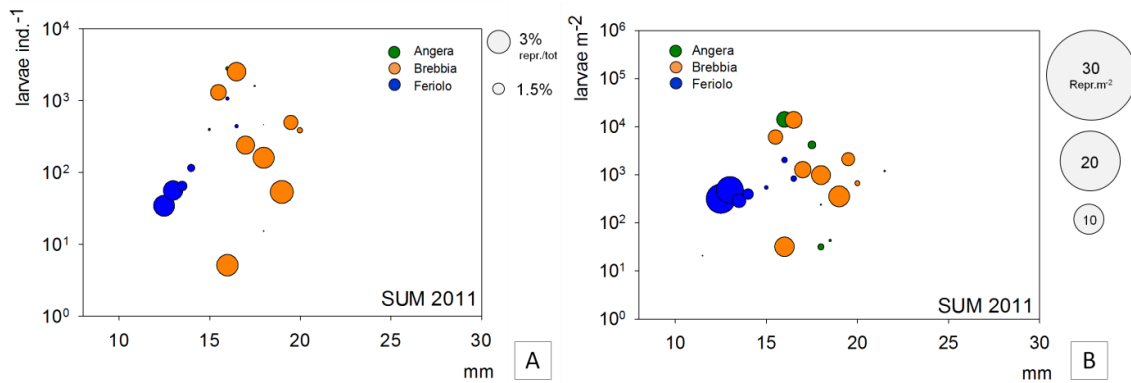


Figure 64: Larvae production over length (size classes 0.5 mm) in Summer 2011. A = individual production, bubble size = reproductive organisms percentage of sampled populations. B = larvae production per square meter, bubble size = reproductive organisms per square meter

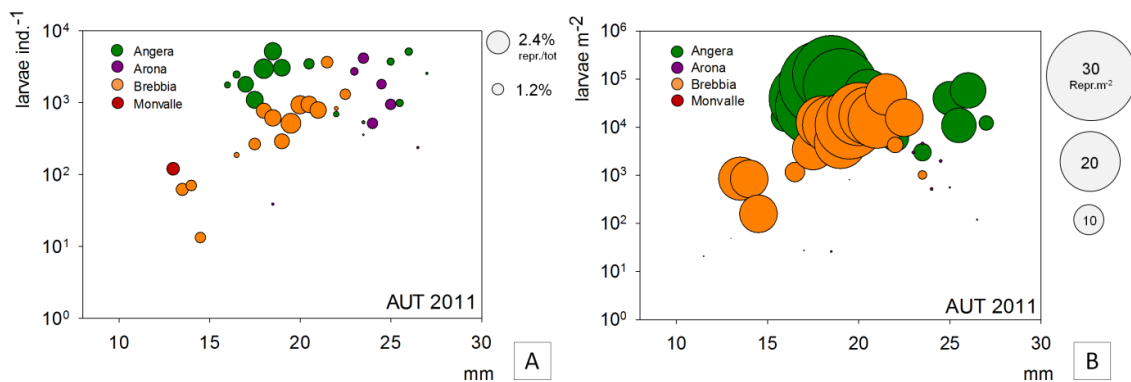


Figure 65: Larvae production over length (size classes 0.5 mm) in Autumn 2011. A = individual production, bubble size = reproductive organisms percentage of sampled populations. B = larvae production per square meter, bubble size = reproductive organisms per square meter

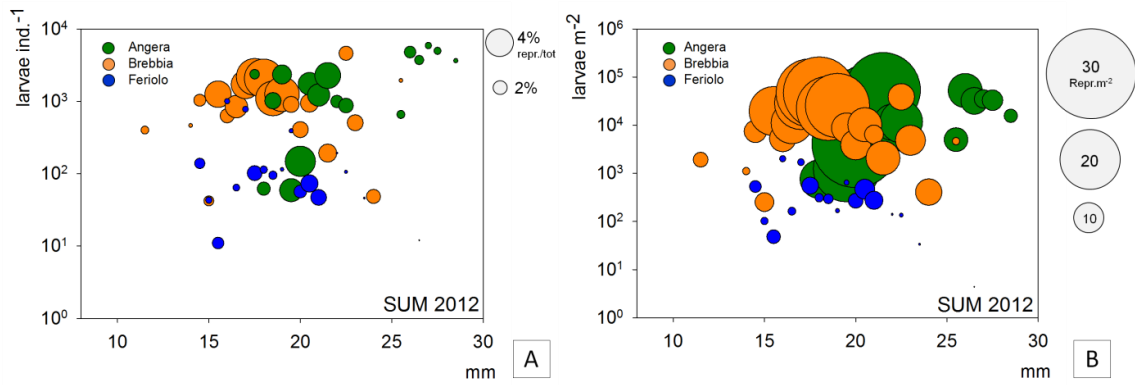


Figure 66: Larvae production over length (size classes 0.5 mm) in Summer 2012. A = individual production, bubble size = reproductive organisms percentage of sampled populations. B = larvae production per square meter, bubble size = reproductive organisms per square meter

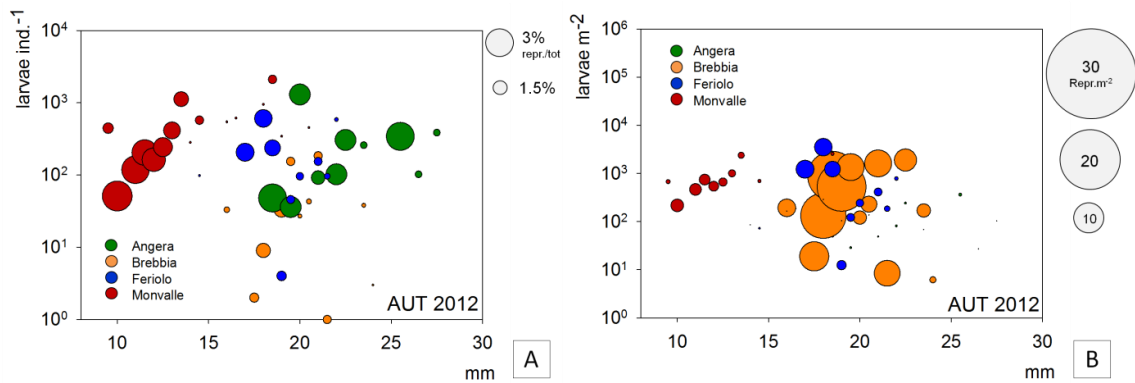


Figure 67: Larvae production over length (size classes 0.5 mm) in Autumn 2012. A = individual production, bubble size = reproductive organisms percentage of sampled populations. B = larvae production per square meter, bubble size = reproductive organisms per square meter

Observing figures from 63 to 67, it emerges that the sites of Angera, Brebbia and Arona present the biggest reproducers in the different seasons.

Angera and Brebbia are also the sites where the highest percentage of reproducers is observed. In those sites it is recorded the production of a high number of larvae (both as individual and per square meter production).

The site of Angera is an exception in the sampling of Autumn 2012, where the production per square meter is strongly reduced, as a consequence of high reduction of population density.

Until Summer 2012 Feriolo presents lower size class of reproducers than at the other stations (except Monvalle). Afterwards population growth is observed with a consequent increase of reproductive classes. In fact, the following Autumn the reproducers reach lengths similar to the ones recorded at Brebbia, however the number of larvae produced is lower to the one of the most productive sites (Angera, Brebbia and Arona).

3.6 Clearance rate

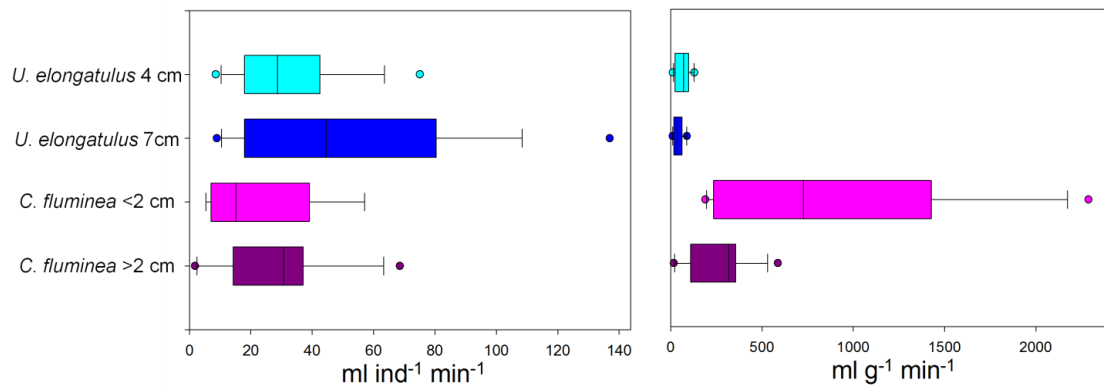


Figure 68: Filtration rates comparison between young and adult organisms of *Unio elongatulus* and *Corbicula fluminea* related to individual (on the left) and tissues dry weight (on the right)

An analysis of the filtration rates for the selected size classes (4 and 7 cm for *U. elongatulus*; < 2 and > 2 cm for *C. fluminea*) demonstrate that younger animals have an individual filtration rate lower compared to older animals.

Moreover *U. elongatulus* has a higher filtration rate than *C. fluminea* (Figure 68).

The filtration rate calculated over one gram of tissue is higher for young organisms; this result is explained by the higher metabolic rate that characterizes the younger animals. In this case, the filtration rate of *C. fluminea* is much higher than the *U. elongatulus* (about ten times higher).

Filtration rates detected are used to estimate water quantity filtered in one square meter in Brebbia site. This site has been selected thanks to the availability of data of density for the analysed species.

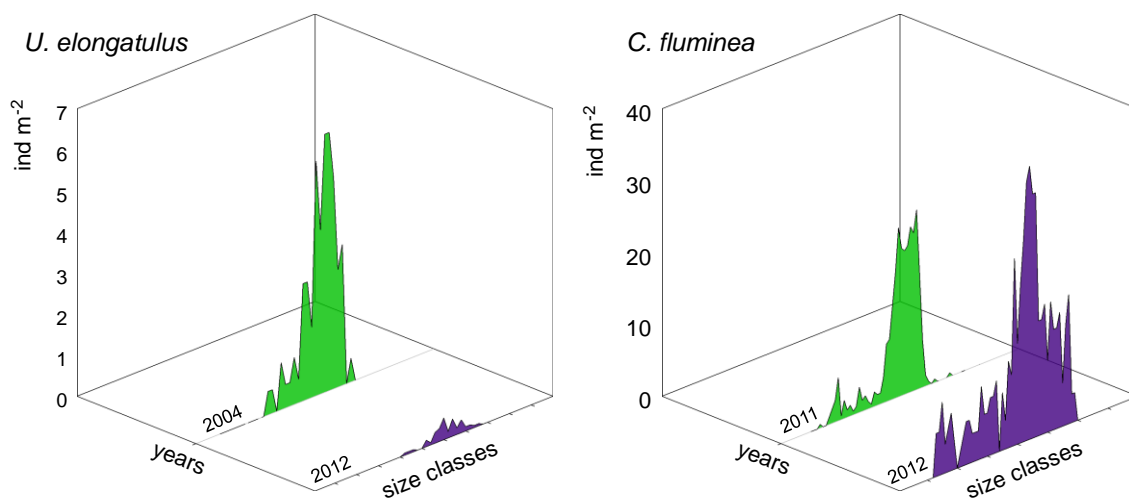


Figure 69: Population density of *Unio elongatulus* and *Corbicula fluminea*
U. elongatulus on left side, size class range = 0.1 mm, years 2004 and 2012
Corbicula fluminea on right side, size class range = 0.05 mm, years 2011 and 2012

Previous data (relative to year 2004) illustrate the population structure of *Unio elongatulus* in sampling site of Brebbia; these data are compared with actual results of sampling performed during Summer 2012 (Figure 69).

Density of size classes appears strongly decreased from 2004 to 2012 (from 47 ind.m⁻² to 2 ind.m⁻² respectively).

Distribution in size classes of organisms not appear to be varied, with a peak around 54 - 63 mm.

Corbicula fluminea is established in Brebbia before 2010; in 2011 population is represented by 1249 ind.m⁻² and in 2012 reaches density of 2418 ind.m⁻².

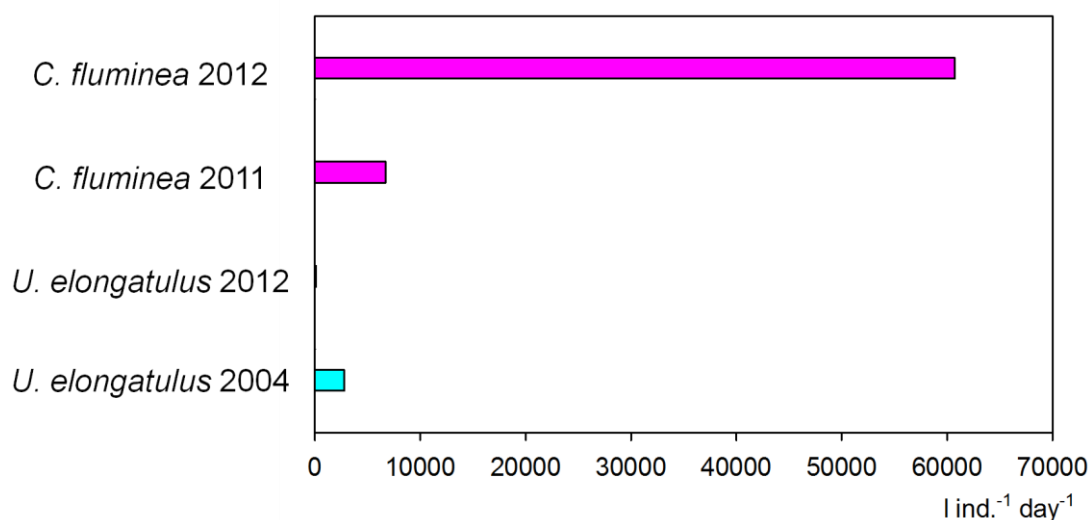


Figure 70: Quantity of water filtered by organisms present in a square meter at Brebbia

Estimation of quantity of water filtrated in a square meter of sediment, depending on the species of mollusc or year considered is illustrated in figure 70. In table 27 are reported as percentages the values obtained: the quantity of filtered water daily by clams in 2012 is considered to be 100%, while the year before filtration rate is 11%. The change is remarkable considering that in 2004 the dominant species, *U. elongatulus*, filtered only 4.6% of what is currently filtered by the sole *C. fluminea*.

	%
<i>Corbicula fluminea</i> 2012	100
<i>Corbicula fluminea</i> 2011	11
<i>Unio elongatulus</i> 2012	0,2
<i>Unio elongatulus</i> 2004	4,6

3.7 Stable isotope analysis

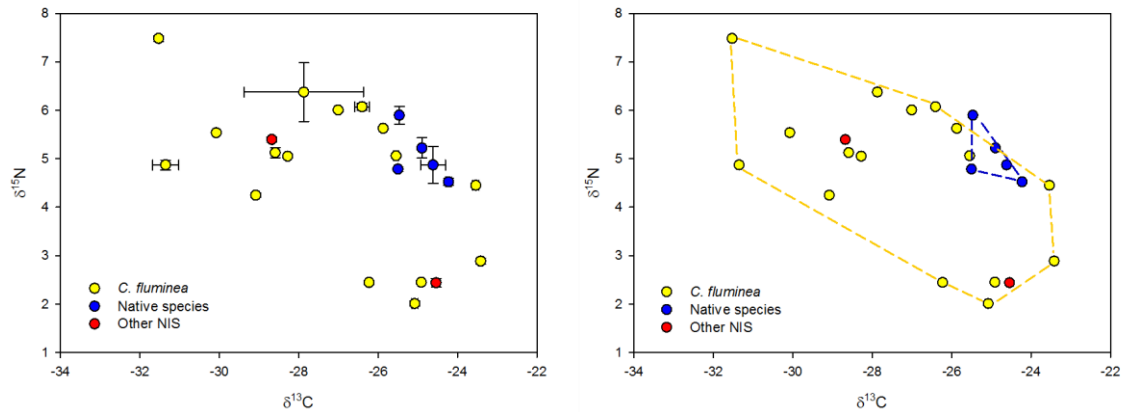


Figure 71: Stable isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of mollusc species. On the left side bidirectional bars indicate standard error. On the right side dotted lines define trophic niches

Stable isotope analysis highlights remarkable variations during the year for *C. fluminea*, which indicates a wide trophic niche, as expected for opportunistic species (Figure 71).

$\delta^{13}\text{C}$ values fluctuate between a maximum of -23.42 in Spring (Brebbia) and a minimum of -31.53 in Winter (Feriolo) reflecting different carbon source assimilated during the four seasons.

Also $\delta^{15}\text{N}$ undergo to wide variations during the year, from 7.48 in Winter (Feriolo) to 2.45 in Autumn (Feriolo).

Width of *C. fluminea* trophic niche, determine an overlapping with the niche occupied from autochthonous species that have similar diets. In fact, observing figure 71 a grouping of native bivalve species can be noted species (*U. elongatulus*, *A. anatina* and *A. cygnea*) with $\delta^{13}\text{C}$ values of -24.94 ± 0.54 and $\delta^{15}\text{N}$ of 5.06 ± 0.56 (Table 28). Variations during the year are small with similar carbon source and a trophic level almost unvaried.

Table 28: Results of stable isotope analysis of bivalves of Lake Maggiore

	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	% C	% N	C:N
<i>A. cygnea</i> and <i>A. anatina</i>	-24,87	4,84	39,56	7,37	5,36
<i>Unio elongatulus</i>	-25,04	5,38	43,08	8,84	4,94
<i>Corbicula fluminea</i>	-27,09	4,67	48,04	9,33	5,22
<i>Anodonta woodiana</i>	-28,68	5,40	49,14	7,15	6,87
<i>Dreissena polymorpha</i>	-24,54	2,44	46,58	10,79	4,32

The more pronounced seasonal variation distinguish young organisms of *C. fluminea*, that seems to be able to use a more wide range of aliments respect adults of the same specie (Figure 72).

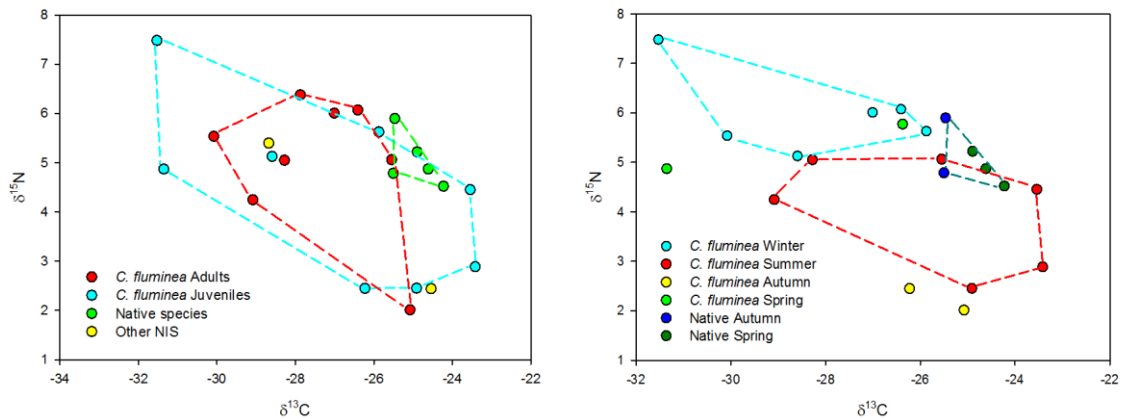


Figure 72: Trophic niches comparison between young and adults organisms (on the left) and seasonal variations (on the right)

In fact, juveniles (with dimension < 10 mm) have a larger trophic niche, which, most likely, determine a higher competitive interaction with autochthone species.

C. fluminea generally indicates higher $\delta^{15}\text{N}$ values in cooler seasons (Winter and Spring) and lower values of $\delta^{13}\text{C}$ in same seasons while in warmer seasons (Summer and Autumn) is possible to observe a less depleted $\delta^{13}\text{C}$ and less enriched $\delta^{15}\text{N}$.

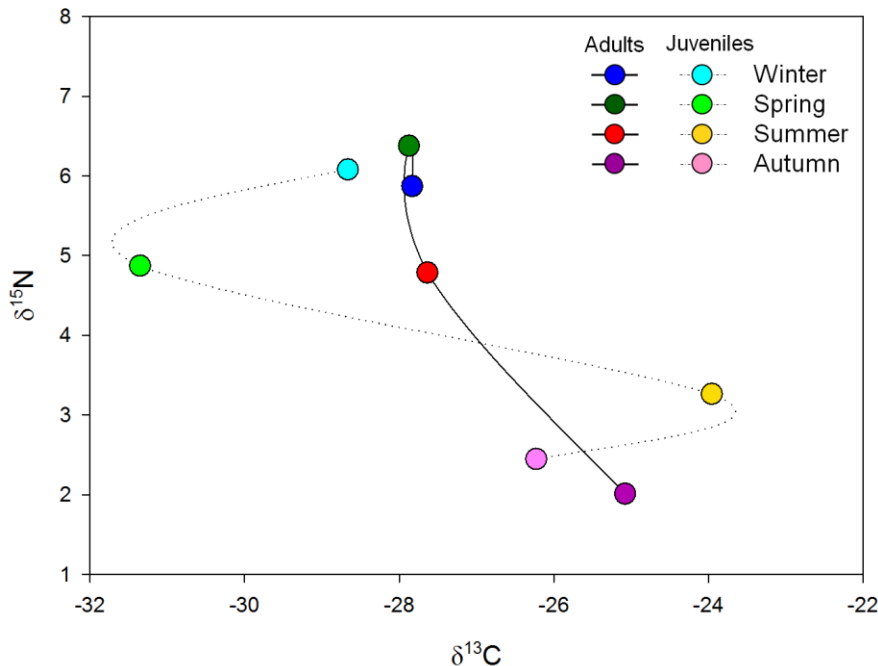


Figure 73: Seasonal variation of young and adults of *C. fluminea*

The variation of nitrogen is similar considering organism with different dimensions/age while carbon presents more variation in juvenile than adult specimens (Figure 73 and Figure 74).

In fact, adults seems to employ similar carbon sources in Winter, Spring and Summer, while in Autumn it is recorder the less depleted value.

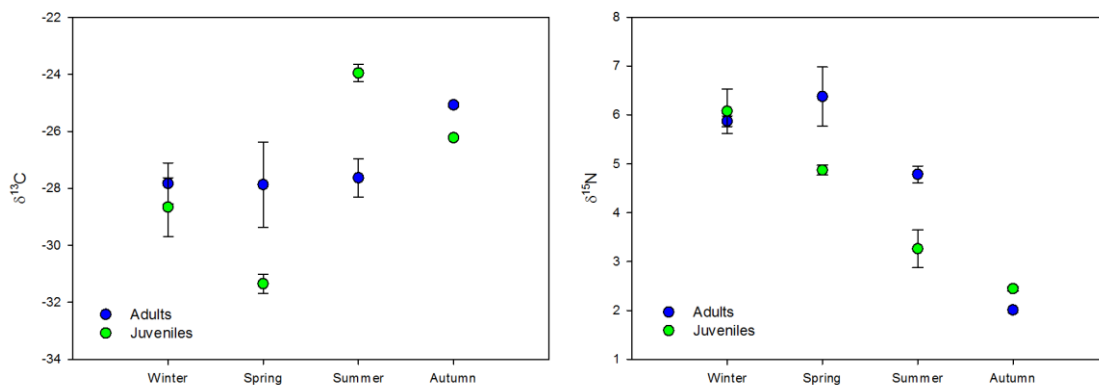


Figure 74: Seasonal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ content in young and adult of *C. fluminea*. $\delta^{13}\text{C}$ (on the left) and $\delta^{15}\text{N}$ (on the right) - Bars indicate standard error

Analysing difference among sampling sites of *C. fluminea* it is possible to observe a similar niche at Brebbia and Arona, while Feriolo has a wide niche. This result may be related to the organic load transported by River Toce water which flow nearby the sampling area (Figure 75).

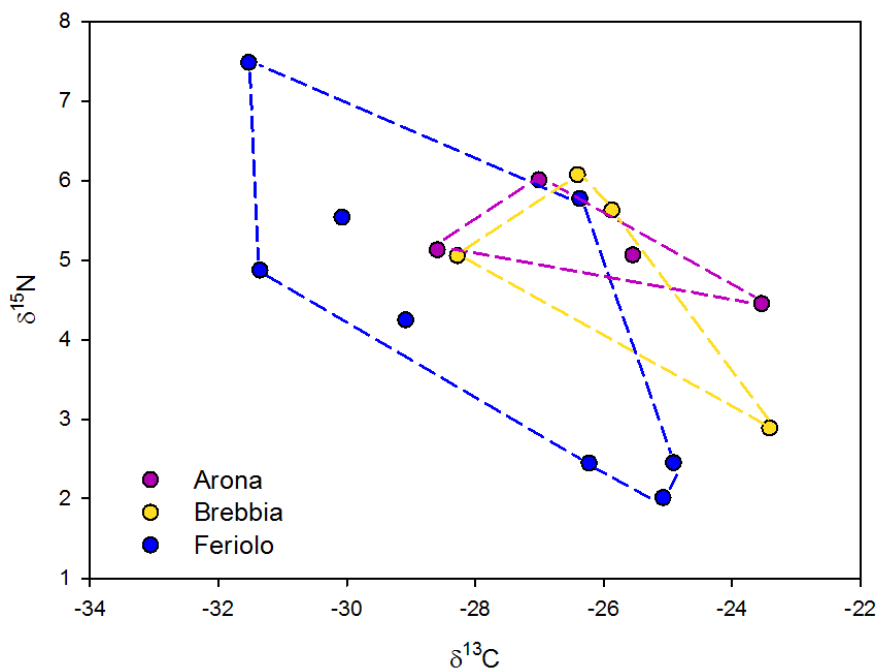


Figure 75: Trophic niches of *C. fluminea* at the selected sites

These data are compared with those of a study which examine some fish species (*Coregonus lavaretus*, *Alosa agone* and *Rutilus rutilus*) and zooplankton species (of genus: *Bythotrephes*, *Cyclops*, *Leptodora* and *Daphnia*) of Lake Maggiore.

From the results of analysis of C fraction it is possible to distinguish the pelagic trophic chain from the littoral chain according to carbon source mainly assimilated from the

different trophic levels, compared with the feeding base: *Daphnia* is considered representative of the pelagic chain and Chironomids and Gammarids for the littoral chain.

The results of the stable isotope analysis divides species tested in pelagic and littoral chain (difference of the carbon source).

Isotopic fraction variations of $\delta^{15}\text{N}$ enable to discriminate trophic levels of the chain (from the prey, lower values, to the predators, larger values).

Bivalves seem to be inserted in the littoral chain, as connecting link among base signal and higher level consumer. For instance, in figure 76 it is illustrated the mean isotopic signal of bivalves in March and November in relation to components of the lake trophic net, from most abundant zooplanktonic species to fish species.

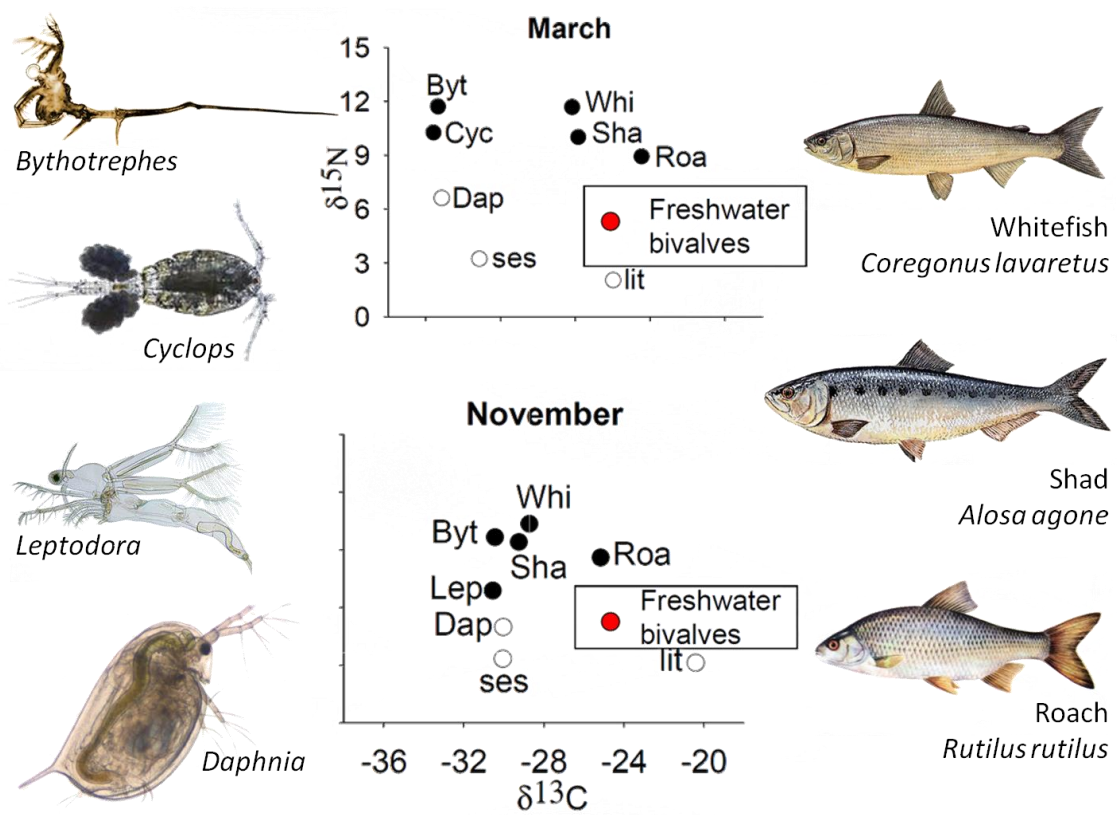


Figure 76: Stable isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of zooplankton, bivalve and fish species of Lake Maggiore (Visconti *et al.*, in press)

In figure 77 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic components variations are reported for *C. fluminea*, that, as observed before, show differences in feeding source used in the different seasons.

Seasonal variations occur also for zooplankton, dividing the analysed species in two large groups: *Daphnia* and *Bosmina* (with a low trophic level), *Bythotrephes* and *Leptodora* (predators at a higher trophic level).

The zooplankton has greater seasonal variation than *C. fluminea*: this result is due to its short life cycle. In fact these animals reflect feed source changes.

Fish have a longer life cycle, the isotopes accumulate over time lead to more stable values.

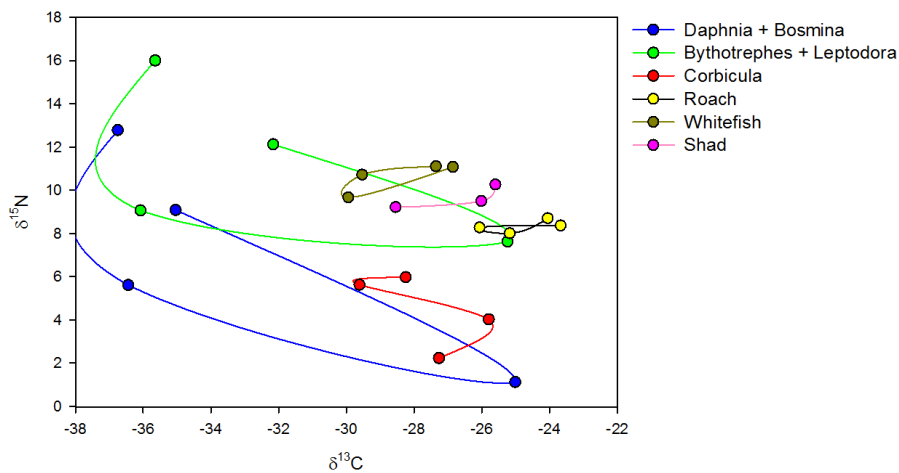


Figure 77: Stable isotopic ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) variation during the year of Lake Maggiore species

3.8 *New parasites*

This part illustrates the presence of parasites accidentally found during development of this study. As a matter of fact, besides field activities, it has been necessary to collect bivalves for lab analysis. Sampled animals for this purpose, are kept in semi-natural conditions for a variable period of time, necessary for the preparation of tests. During this time emerged the presence of parasites.

3.8.1 *Digenea Bucephalidae*

In Summer 2011 some aquariums were prepared to evaluate the filtration rate. Some bivalves, belonging to genus *Unio*, are disposed in some tanks to transfer them from the pool to the aquariums.

The conditions to which animals were subjected during the preparation phase, caused an unexpected release of numerous small swimming organism inside the tank.

Observation of these organisms determines identification of cercariae belonging to Bucephalidae family (Digenea: Trematoda; Poche, 1907; Figure 78).

Generally speaking, trematods are internal parasites of molluscs organs and of vertebrates (Overstreet *et al.*, 2002). In molluscs the parasites attack tissues and gonads, while in vertebrates are present characteristically in gut, but also anchored to body-cavities, organs and tissues, while rarely are ectoparasitic under fish scales. Bucephalidae are easily found in all aquatic environments, in Italy they are recognized for marine waters (Špakulová *et al.*, 2002; Bartoli *et al.*, 2005), but a few papers described their presence in inland lentic and lotic waters (Galli *et al.*, 2001, 2003).

The origin of these parasites is Eastern Europe and Western Siberia (Bauer, 1987), but the same occurred in Western Europe, especially in France (Wallet *et al.*, 1985; Molloy *et al.*, 1997), and recently in Central Europe, Austria (Mühlegger *et al.*, 2009).

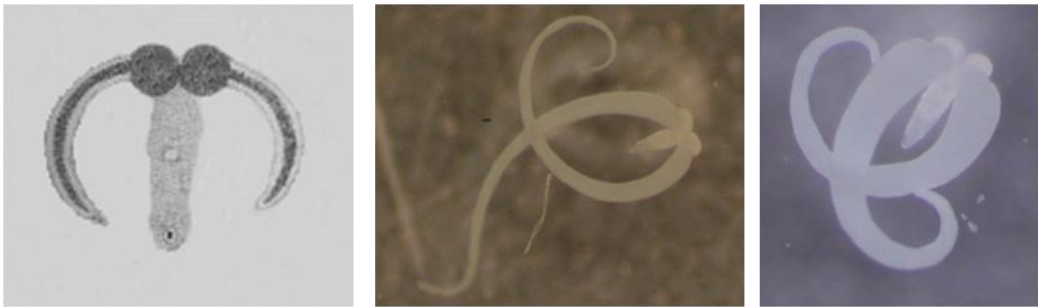


Figure 78: Historical picture (modified from Badcock, 1875) and images of digenean cercariae parasites swimming in the water

At the early beginning and for a long time only one name was proposed among Bucephalidae, *Bucephalus polymorphus*, and many different freshwater species were ascribed to it, confusing it with a different genus *Rhipidocotyle*. After the works of Baturó (1977, 1978, 1980) it was discovered that this name belongs only to the cercariae developing in the mantle cavity of *Dreissena polymorpha*, while those derived from Unionids belong to *Rhipidocotyle campanula* (= *R. illense*) (Ziegler, 1883) (Baturó, 1977). In fact, although there are numerous reports of *Anodonta* sp. and *Unio pictorum* (Unionidae) infected with *B. polymorphus*, many authors (Baturó, 1977; Molloy *et al.*, 1997; Karatayev *et al.*, 2000) prove that there is a narrow specificity to the first intermediate host.

The life cycles of these two parasites are almost similar (Figure 79) and involve three different hosts: the first intermediate host a mollusc, the second intermediate host a cyprinid fish and the final host a predatory fish. *B. polymorphus* infection commences in zebra mussel entering the mussel's visceral mass, primarily affecting the gonads, causing *Dreissena* sterility (Grizzle & Brunner, 2007). From the gonads, at the end of the development, the cercariae exit from the mantle cavity and enter the second intermediate host across the water. Within cyprinids the cercariae become metacercariae and move into the intestine of the final hosts (various piscivorous fishes) when eating infected fish (Molloy *et al.*, 1997). Mature specimens are characteristically found in the intestine of predatory fish as *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Alburnus alburnus*, *Tinca tinca*, *Gobio gobio*, *Sander lucioperca*, *Perca fluviatilis*, and sometimes *Esox lucius* (Niewiadomska, 2003). The highest number of *B. polymorphus* cercariae emerge among June and September from *D. polymorpha*, and should have a high pathogenicity penetrating the fish body and inducing the death of the fish due to hyperaemia and haemorrhage, muscle deformation of the vertebral column, liver necrosis, parenchymal kidney damages (Baturó 1977, 1978, 1980; Grizzle & Brunner, 2007).

In Europe there are two species of *Rhipidocotyle* in the Unionid *Anodonta anatina*: *Rhipidocotyle campanula* and *Rhipidocotyle fennica* (Gibson *et al.*, 1992). In this case, the

first intermediate host should be an Unionids, while the second intermediate host a cyprinid fish like *Rutilus rutilus*, *Scardinius erythrophthalmus*, or *Perca fluviatilis* (Taskinen *et al.*, 1991; Gibbson *et al.* 1992). The entry in the final host is passive, because cercariae act like planktonic organisms entering their host through the gills or the mouth and inducing gills and brain hyperaemia with haemorrhage, eyes bulb hyperaemia, destruction of the muscular fibres, liver necrosis, parenchymal kidney damages. The infection is more pronounced respect to the *B. Polymorphus* one (Grizzle & Brunner, 2007).

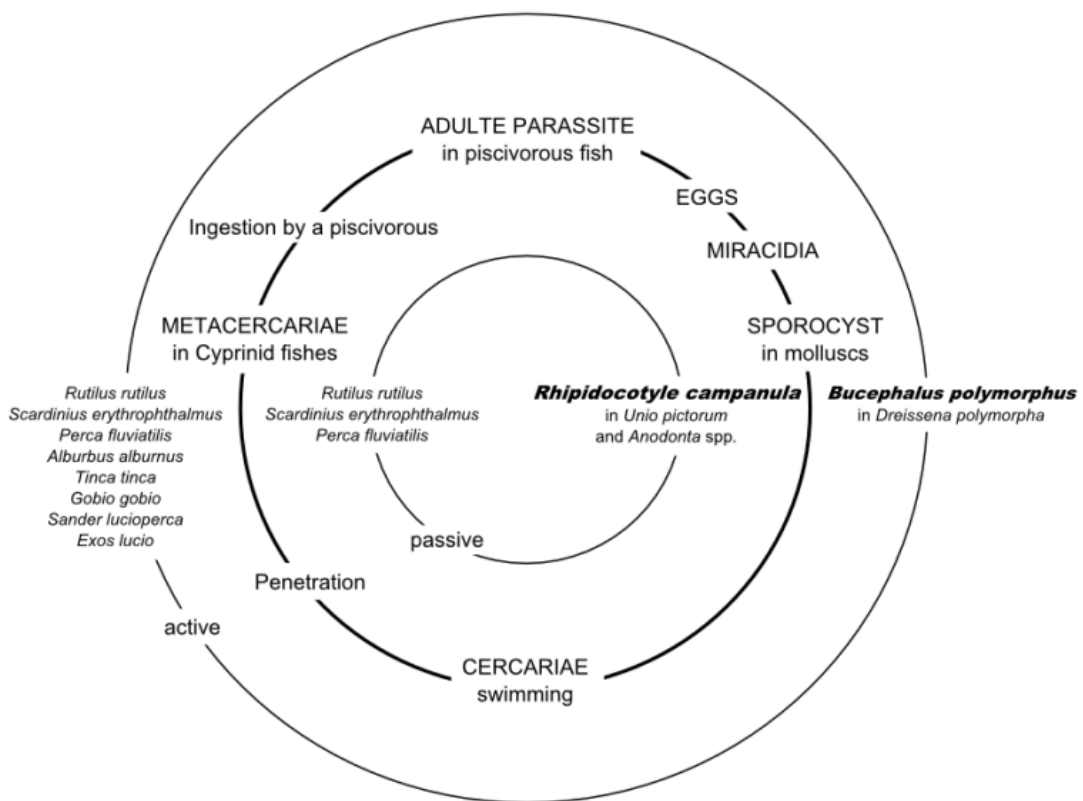


Figure 79: Life cycle of trematods digenea (modified from de Kinkelin *et al.*, 1969)

3.8.2 *Rhodeus amarus*

In Summer 2012 in the pool are present organisms of different species (*C. fluminea*, *A. woodiana*, *U. elongatulus*, *A. anatina* and *A. cygnea*) collected in Lake Maggiore. In this period some small specimens of a fish species appear inside the pool. It is the *Rhodeus amarus* (Bloch, 1782), a group of freshwater fishes that belong to the subfamily Acheilognathinae (family Cyprinidae), which have relatively small length, rarely exceeding 70 mm (from the tip of the snout to the origin of the caudal fin) (Smith *et al.*, 2004; Liao *et al.*, 2013).

During the spawning season, bitterlings display sexual dimorphism: males develop a pink flush on their ventral and lateral sides, becoming almost red near the pectoral fins. Upon reaching sexual maturity the dorsal section of the iris is red and remains pigmented throughout life (Figure 80).

The colour of the females remains unchanged during the spawning season: grey-green dorsally, silvery laterally and yellowish ventrally, and without red pigment around the iris (Smith *et al.*, 2004).

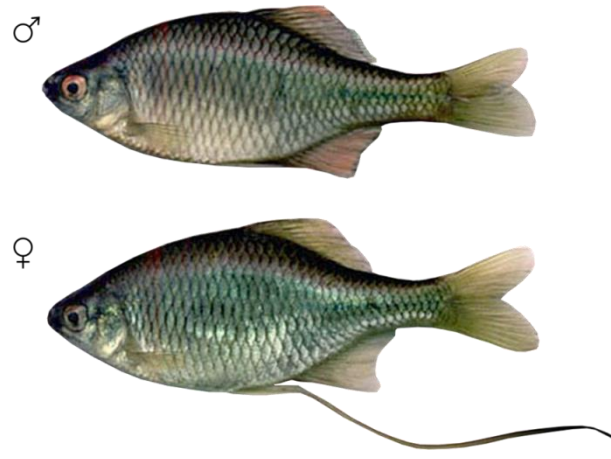


Figure 80: *Rhodeus Amarus*
Modified picture collected from web:
www.ittiofauna.org; Original photo by Artaev, O.

European bitterling have a resource-based mating system; females lay their eggs in the gills of living freshwater mussels. During the reproductive season (April to June, with a peak during a three-week interval in late April–May), males bitterling compete aggressively for mussels and successful males establish territories in the immediate proximity of mussels (single or a cluster of several), court females and lead them to their territories to spawn (Konečná, 2009).

It is also common in bitterling the sneaking behaviour, in which a rival male (an adjacent territory holder or a male that does not possess a territory) releases his sperm into a rival's mussel (Casalini *et al.*, 2009).

Bitterlings display morphological, physiological, and behavioural adaptations for using mussels as spawning sites. Females develop long ovipositors that they use to place their eggs onto the gills of a mussel through the mussel's exhalant siphon. Males fertilize the eggs by releasing sperm into the inhalant siphon of the mussel, so that water filtered by the mussel carries the sperm to the eggs (Figure 81; Smith *et al.*, 2004; Liao *et al.*, 2013).

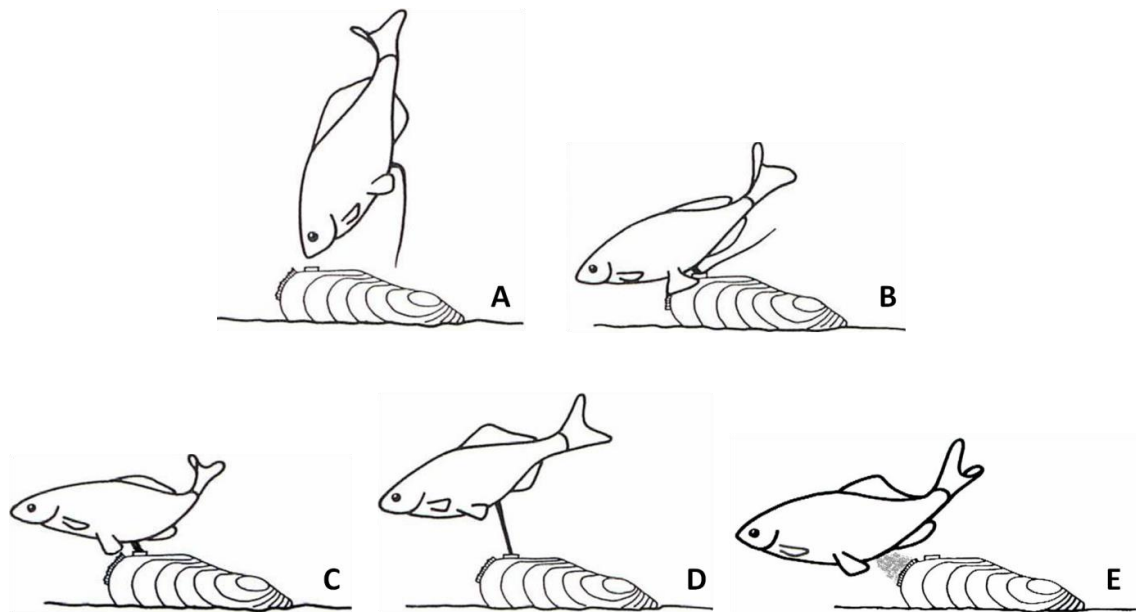


Figure 81: *Rhodeus amarus* - eggs deposition and fertilization

A – Female bitterling choice of mussel

B - Ovipositor insertion in exhalant siphon

C, D – Eggs deposition and siphon extraction

E - Sperm release in the inhalant siphon

Modified from Smith *et al.*, 2004

The bitterling mating system is promiscuous; both males and females spawn repeatedly, with multiple partners. Female oviposition decisions are based on both male and mussel quality.

Females deposit one to five (typically three) eggs in the mussel gill chamber. Females spawn in several bouts lasting one or two days and consisting of approximately 5–10 independent spawning each day (Casalini *et al.*, 2009).

Eggs are large and elliptical; a response to Unionid gill morphology and a way of increasing the transfer rates of respiratory and excretory products to and from the tissues. The yolk sac develops elongated lateral processes during early ontogeny; these secure the embryo into the host's interlamellar space. Once the larva is capable of movement the lateral processes are lost and the larva becomes to be less dependent of the host's gills for the provision of oxygen. Bitterling larvae generally emerge from the host once the yolk sac has been consumed, after a period of time of 4-6 weeks, depending on temperature. Sexual maturity is reached by both sexes in 1 year, when the fish are 30–35 mm standard length (Aldridge, 1999; Smith *et al.*, 2004).

Rhodeus is the only bitterling genus presents in Europe. It is distributed from the Neva basin (Russia) to the Rhoden basin (France) and southward to the Black Sea. His native area covers roughly the present distribution, although in Eastern Europe is linked to the increase carp cultivation during the middle Ages. In Italy, this species was accidentally introduced consequently to a restocking of cyprinid species. The first record dates to 1990 in the area of Verona (Northeast of Italy). In the following 20 years the species spreads northward reaching the lakes of South Tyrol and westward, following the course of the River Po (Libertini *et al.*, 2008).

Results of this study show that the Lake Maggiore offers a favourable environment for the establishment of the Asian clam *C. fluminea*. In the selected sites populations have considerable abundances, high growth and reproduction rates. In addition, the colonization of the littoral zone demonstrates an active northward spread; in fact it was possible to observe the phenomenon in the *lag* phase for the site of Monvalle. Finally emerges a niche overlap with the native Unionids species, which are strongly declining in the last decade in this environment.

The picture that emerges from the study of *C. fluminea* populations is quite complex, with differences among the sampling sites. The analysis of the biometric features highlights significant differences in shape among the organisms collected at the five sites. The differences in shape may be due to the density of the populations, to individual variability or to environmental conditions (Hendelberg, 1961; Kesler & Bailey, 1993). Variations in the shape due to different environmental conditions are reported for *A. woodiana* and other species of Unionids (Zieritz & Aldridge, 2011; Soroka & Zdanowski, 2001; Ravera *et al.*, 2007).

The shape differences detected by statistical analysis are not related to density, in this case sites with higher densities (Angera and Brebbia) and sites with low densities (Arona Feriolo Monvalle) would present similar shapes.

On the contrary, similar shapes are found at sites of Angera and Arona; this similarity can be explained by representative size classes of the population. Larger size classes are well represented in Angera and Arona, while are almost absent in Feriolo and Monvalle.

Small size classes are almost entirely absent in the population of Arona. The differences in dimensional structures of populations are the factor that most influences the observations, while density and environmental factors seems to be less evident.

The populations of the different sites also differ in length/weight ratio. The organisms of size classes < 12 mm have a length/weight ratio which varies in a similar manner in the five sites, while with the increase of the size differences among the populations are higher. In particular, the curves of the population of Arona have a lesser slope, and therefore specimens have a lower weight for the same length in comparison with the populations of the other sites. The main causes that can explain the phenomenon are limited food source, high reproductive rate and environmental conditions. However the availability of food can be excluded from the possible causes as Arona has a high organic load. Observations on the larval production of this site lead to disprove the second hypothesis. The environmental conditions of this site are not optimal: anoxic sediment, presence of discharge and waste of all kinds; this is the reason why this seems to be the most likely hypothesis. The differences highlighted by the statistical analysis for the site of Monvalle can be attributed to the small size of the organisms that compose this population.

The density and biomass observed in several sites reach higher values in Angera and Brebbia, lower values are found in Feriolo. The populations of Arona and Monvalle have

the density and biomass significantly lower. Different environmental characteristics of Arona than the other sites examined seem to limit the growth of the population, in agreement with what described by Ilarri et al. (2010). For Monvalle these values are due to a population composed of individuals of small dimensions, just established and therefore still in the phase of acclimatization (lag phase), the following phase is characterized by exponential growth (log phase) observed in subsequent samplings (Nentwig & Josefsson, 2010).

At the various sites is observed trends common: the density is generally higher in the warm seasons (Summer and Autumn), while there is a decrease in Winter similar to that reported in literature (Werner & Rothhaupt, 2008).

In general, the density and biomass of *Corbicula fluminea* observed are rather high, with peaks reaching up to 3000 ind.m⁻² and 2500 g m⁻², values significantly higher than those found in other Italian ecosystems, as the river Ticino (Nicolini & Loyola, 2011). These data are in accordance with the literature, which reports an achievement of high densities for *Corbicula* (Sousa et al., 2008b; Sinclair, 1971; Stites, 1995). Similarly to what found in the literature, the highest values of density and biomass were recorded in sites characterized mainly by fine sediment and sand (Sousa et al., 2008b; Bagatini et al., 2007). The size structure of the populations provides clues to understand the process of invasion and its phases. Differences in density and dimensional structures of populations are useful not only to assume the dynamics of invasion in the new environment, but also provide information on environmental conditions (Boltovskoy et al., 1997). Abiotic conditions that may affect the development of the population include the redox potential, the concentration of nutrients and calcium, the hardness of water, the organic matter, the characteristics of the sediment and temperature variations (e.g. Sousa et al., 2008a, 2008c). The site of Arona has a very fine sediment, not very suitable for *Corbicula*, which prefers sandy substrates that characterize other sampling sites (Cordeiro & MacWilliams, 1999; Paunović et al., 2007; Sousa et al., 2008b). Coarse sediments allow better water circulation and consequently better oxygenation. At Arona the sediment appears dark, index of anoxia, a condition poorly tolerated by *Corbicula* (McMahon, 2000).

The populations size structure in the sampling sites show large differences, indicative of a different demographic dynamics. Already in 2010, at the time of the first discovery of this species in Lake Maggiore (Kamburska et al., 2013a), populations of Angera, Arona and Brebbia were characterized by a multi-modal frequency distribution, while in the site of Feriolo it has been registered an unimodal distribution (left skewness). The southern sites (Angera and Arona) host organisms with higher length/age (there are animals with length > 26 mm and an age of about 3 years). The organisms collected in Brebbia have an estimated age ≤ 2 years while Feriolo organisms have small length/age (≤ 1 year). The timing of colonization of Lake Maggiore follows this order: Monvalle <Feriolo <Brebbia <Angera <Arona. It is also possible to estimate that the colonization of this environment began in 2007 (Kamburska et al., 2013a), for expansion of the population already established in the effluent, the river Ticino (Nicolini & Lodola, 2011). The application of the Kolmogorov-

Smirnov test confirms the dynamics of colonization proposed, find significant similarities between the distributions of Angera and Arona, but also between the distributions of Feriolo and Monvalle. The timing of colonization may explain the presence of larger animals collected at the sites of Angera and Arona.

The populations size structure highlights reproductive events, which are usually observed in the warm seasons (Summer and Autumn), with the exception of Winter 2013. In this period is observed high presence of organisms with length < 5 mm in all sampling sites. The recruitment of new organisms is scarce at the site of Arona, while the opposite situation is observed in Feriolo.

The reproductive period of *Corbicula* in Lake Maggiore showed a single reproductive peak from late Spring to Autumn. A similar observation was made by Doherty *et al.*, (1987) and by Mouthon & Parghentanian (2004). Contrary observations are published by Cataldo & Boltovskoy *et al.* (1998) in the delta of the Parana River (Argentina) and Sousa *et al.* (2008a) that report 2 reproductive events per year.

The number of larvae produced by each adult is highly variable, with values ranging from a few hundred to 8000 larvae per demibranch, values significantly higher than those found by other authors (Denton *et al.*, 2012; Byrne *et al.*, 2000).

At the sites of Angera, Arona and Brebbia is observed a high individual production, while in Feriolo and Monvalle individual production is very low. A high percentage of reproductive organisms is found in site of Angera and Brebbia, while Feriolo and Monvalle populations, composed predominantly by small size organisms, show a lower percentage. Arona environmental conditions are responsible for the low densities found, which entail a low larval production per area unit (m⁻²).

The minimum reproductive size is reached around 10 mm of length. At Monvalle were observed smaller reproductive individuals (approximately 8 mm), this site is of recent colonization, it is possible that organisms anticipate the attainment of reproductive size. Reproductors of similar size are observed even by Sousa *et al.* (2008a). The larvae are released into the environment when they reach the complete development, generally have a length of 250 µm, as also indicated by Sousa *et al.* (2008a).

The Kolmogorov-Smirnov test applied to distribution frequency of the population does not highlight a selective size class mortality.

Generally in sites Angera, Brebbia, Feriolo and Monvalle mortality is rather low, while at Arona is considerably higher. At Angera was observed a phenomenon of mass mortality in Autumn 2012. Phenomena of mass mortality are reported for this species (Vohmann *et al.*, 2010; Werner & Rothhaupt, 2008; Ilarri *et al.*, 2010) and affect the population regardless of the phase in which they are. Usually these phenomena are related to particularly harsh Winters or to the decrease of the dissolved oxygen concentration that is observed with a strong lowering of the water level accompanied by an increase of the temperature (Werner & Rothhaupt, 2008; Ilarri *et al.*, 2010). In the site of Angera these factors were combined, in fact, the mass mortality affected the population in late Summer-early Autumn, in conjunction with a greatly reduced water level and excessive temperature. This site also

has a particular conformation: the slope increases rapidly just a few meters from the shore; *Corbicula* is therefore confined in a small area. In the other sites the slope is gentler and when the water level decreases populations are dispersed in a wider area. For this reason phenomena of mass mortality were not been observed as instead occurred in the site of Angera.

An analysis of the dimensional structure of the Angera empty shells after mass mortality allows to assume that the shells remain in the environment for a rather long period of time, especially for larger organism shells. In fact, the shells of the small size organisms, thinner and fragile, are degraded more rapidly. The oundation (which has variable intensity among sites) promotes the transport of shells towards the shore, changing the ratio of live animals and empty shells collected. Living organisms usually are buried in the sediment and suffer less wave action, while the shells are lighter and unable to oppose the water movement. Moreover the transport promote the degradation, then in the sites subject to greater oundation the shells will persist for a shorter time.

The size structure of the populations also reveals an increase in the size of cohorts sampled, indicating the organism growth. *C. fluminea* has a high growth rate, which is limited by some environmental factors such as salinity, temperature, pH, dissolved oxygen concentration, availability of calcium and food (Lucy *et al.*, 2012). However, none of these factors is limiting the growth and spread of this species in Lake Maggiore. The growth rates are affected by seasonal variations in temperature: in Summer and Autumn organisms show higher values than the other seasons (almost 2 times higher). The growth rate is higher for small size organisms, as expected comparing of young and adults organisms of the same species. Growth rates are described by sigmoidal curves, as described in other studies (Cerrato, 1980). The highest values are found for the populations of Angera and Brebbia.

Applying von Bertalanffy equation variations in the values of the growth rate (k) and in the maximum attainable length (L_{∞}) among sites are observed. These data show a different growth rate that reflects the seasonality and the different environmental conditions to which the populations are subjected (Cataldo & Boltovskoy, 1998; Anthony *et al.*, 2001; Hull *et al.*, 2004).

The identification of the cohorts was not simple and immediate: the observation of frequency histograms of the sampled populations were enough to isolate the younger cohorts. The cohorts constituted by bigger organisms are overlapping and increase the uncertainty of the peak resolution. Two different methods are used to decrease the uncertainty of the observations that emerge from frequency histograms: the method elaborated by Walford (1946) and the one proposed by Harding (1949) that uses the probability paper. However, the application of these methods leads to an ambiguous determination of the cohorts. The Walford method leads to the identification of a cohort number often superior to what hypothesized, especially for populations composed by small size classes organisms. The probability paper understated the number of cohorts due to

difficult interpretation of inflection. So for the estimation of the field growth rate are considered only cohorts uniquely defined and which are observed in subsequent samplings. The differences found in the estimated field growth rate, compared to those measured in semi-natural conditions can be explained by the different conditions to which animals are exposed. In particular, the temperature seems to play a key role. In fact the values observed in the field are also generally some degrees higher than those measured in the pool especially in the warmer seasons (Summer and Autumn) and growth rates are from 6 to 8 times higher. The growth rate has a minor variation in Winter (about 1.2 times higher than in the cage).

In addition to the temperature difference, there are other factors that act on the cage growth rate, such as the effect of the confinement of the organisms in the cages and the consequent reduced food supply (Hull *et al.*, 2004). Reduced availability of food is also attributable to the fact that pool water is pumped from a depth of 20 meters, probably less rich in nutrients than the water that laps the littoral zone. Finally, it can be assumed that the weekly manipulation of animals and cages have added another stress element.

Analysing the results of the filtration rates and isotope analysis is possible to assume a competition between native and alien species in the Lake Maggiore. The results of the filtration rate show higher values for the organisms of smaller size/age, due to a higher metabolic rate that characterizes younger organisms. The individual and per gram of tissue filtration rates are very variable between different species (1.6 times higher for *U. elongatulus* in the first case and from 10 to 18 times greater for *C. fluminea* in the second). Lauritsen & Mozley (1983) report filtration rates with a comparable range with the one estimated for the *Corbicula* of Lake Maggiore (278-607 ml g⁻¹ h⁻¹ in the first case and 263 to 858 ml g⁻¹ h⁻¹ for the Lake Maggiore). Buttner & Heidinger (1981) confirm the values which vary from 300 to 800 ml g⁻¹ h⁻¹. In literature are reported values with significant differences (Sylvester *et al.*, 2005). In some cases the differences are due to the experimental conditions, such as temperature or food composition. Indeed *Corbicula* is able to select the particles by size, shape and quality, rejecting those unwanted (Buttner & Heidinger, 1981; Basen *et al.*, 2011).

As filter-feeders, variations in the shellfish community can lead to considerable changes in the base of the food web of environments (Phelps, 2005; Kryger & Riisgård, 1988; Ricciardi & MacIsaac, 2011). Phelps (2005) lists a number of examples of shellfish effects on the water transparency, for example, to the north of San Francisco Bay, resulting in the introduction of *Potamocorbula amurensis*. A rise of water transparency is also shown for the region of the Great Lakes and the Hudson River as a result of the *Dreissena polymorpha* introduction, in the same way as for the introduction of *Corbicula fluminea* in the Potomac River.

The feeding sources of native and alien species of Lake Maggiore can be assumed by looking at the results of the analysis of stable isotopes (Tykot, 2004). *Corbicula* presents a wider trophic niche than native, as also reported by Atkinson *et al.*, (2010). The major differences in food sources of this species indicate that *C. fluminea* has lower trophic

fidelity, and is therefore able to use a wider range of food sources than the native species. The availability of the food undergoes seasonal variations in Lake Maggiore (Visconti & Manca, 2011) and *Corbicula* is able to adapt to these changes easier than native species. Furthermore, the data show a wider trophic niche for younger organisms of this species. In the literature (Naddafi *et al.*, 2009) is observed for young organisms of *Dreissena polymorpha* a greater demand for nutrients, due to the higher metabolic and breathing rate than adults. A higher demand for nutrients could result in a lower selectivity towards food for the young of *C. fluminea*. Moreover the bivalves seem able to bypass the limitation of nutrients accumulating N and P in tissues. This mechanism, together with the low trophic fidelity, contributes to the success of invasive species (Naddafi *et al.*, 2009).

The decline observed for the native species of Lake Maggiore (*U. elongatulus* abundance decreases about 90 % from 2004 and 2012) is due to several factors. Invasive species present in the environment occupy similar habitats, showing preference for sandy substrates. In particular *C. fluminea* competes with native species for trophic niche. Considering the abundance and the filtration rate of this alien species, it is possible to assume that this invasive species imply a disturbance effect on native species. The entrance of the Asian clam in the lake dates plausibly to 2007 (Kambuska *et al.*, 2013a) so it cannot be the sole cause of the native species decline. Another factor is identified in the establishment of another alien species: *A. woodiana* (Kambuska *et al.*, 2013b). Like other Unionids the life cycle of this species involves in the release of glochids that need a host to complete their development. *A. woodiana* has a low selectivity towards the host and has an earlier reproductive period than the native species. The fishes that have already been infested develop an immune response that protects them from future infections (O'Connell & Neves, 1999). These features cause a reduction in the reproductive success of native species. Reproduction is also affected by changes in fish communities due, for example, to the modification of habitats, or to the introduction of several alien species (Volta & Jepsen, 2008; Riccardi & Rossetti, 2007; Visconti *et al.*, 2011; Casale & Brambilla, 2010; Camusso *et al.*, 2001). The literature reports that areas with high biodiversity are less susceptible to invasion by alien species, and compromised environments offer more possibilities for the establishment of new species (Maron & Vilà, 2001).

The specimens that moving from one environment to another can bring with them their parasites, and also they can find the conditions necessary to establish a new population (Wittenberg & Cock, 2001).

In the development of this study two new species of parasites of Unionids (cercariae of *Bucephalidae* and specimens of *Rhodeus amarus*) have been found. The infection of the gonads due to cercariae leads to the castration of bivalves and can be fatal, while the presence of the larvae of *R. amarus* in the gills reduces the respiration and filtration rates.

The presence of *D. polymorpha* is recognized in the lake since the early 2000s (Camusso *et al.*, 2001) and it is often found anchored to the shell of other bivalve species. This epibiotic behaviour brings a significant advantage to *Dreissena*, which saves energy by

exploiting the moving of water generated by the host. However the number of attached *Dreissena* often becomes too high, causing the death by asphyxia (Sylayeva *et al.*, 2012). The combination of all these factors provides sufficient explanation for the observed decline of native species, however, the invasion of *C. fluminea* appears to be a worrying phenomenon. After 3-4 years from the entry into the lake had colonized a third of the shoreline (Kambuska *et al.*, 2013a). To date, the presence of this bivalve is found in all suitable habitats distributed along the entire perimeter of the lake (Riccardi pers. com.)

Conclusion

The rapid spread of *Corbicula* along the littoral area and its high densities, suggest its potential to become a keystone littoral species. Recreational and fishermen boats in the lake, often accessing from tributary rivers (Ticino, Toce), contribute to species diffusion. Many biological invasions do not occur as a gradual expansion along a continuous front, but result from the expansion of satellite populations that become established at ‘invasion hubs’ (With, 2002).

The decline of native bivalves is likely due to different causes, and *C. fluminea* invasion is one of these. In fact, growth and reproduction rates are very high for this alien species and provide an advantage in habitat recolonization after seasonal mass mortality, these characteristics promote the replacement of the native. In general, mortality have a negligible effects on *C. fluminea* populations. In this environment there are no factors able to limit the growth and spread of *Corbicula* and still there is no evidence about possible predators on in the lake.

Another disturbance element recognizable for native species decline is the establishment of *A. woodiana* that occurs during the last decade. In addition, during this study, the presences of two parasite still unknown in this environment was discovered.

According some authors, the invasions of alien species are responsible of strong changes at different levels within the ecosystem, and cause a reduction of biodiversity. Even though preventive measures are to be preferred, methods to control already established invaders can be applied at least to avoid further dispersal to still uninvaded systems. Eradication is generally impossible – and expensive whenever possible – and can have negative impacts on native species.

Methods to control or restrain *C. fluminea* diffusion should be focused not only on reducing its density and thus moderating its impacts on the ecosystem, but also to reducing the opportunity for *C. fluminea* to further dispersion across Lake Maggiore basin.

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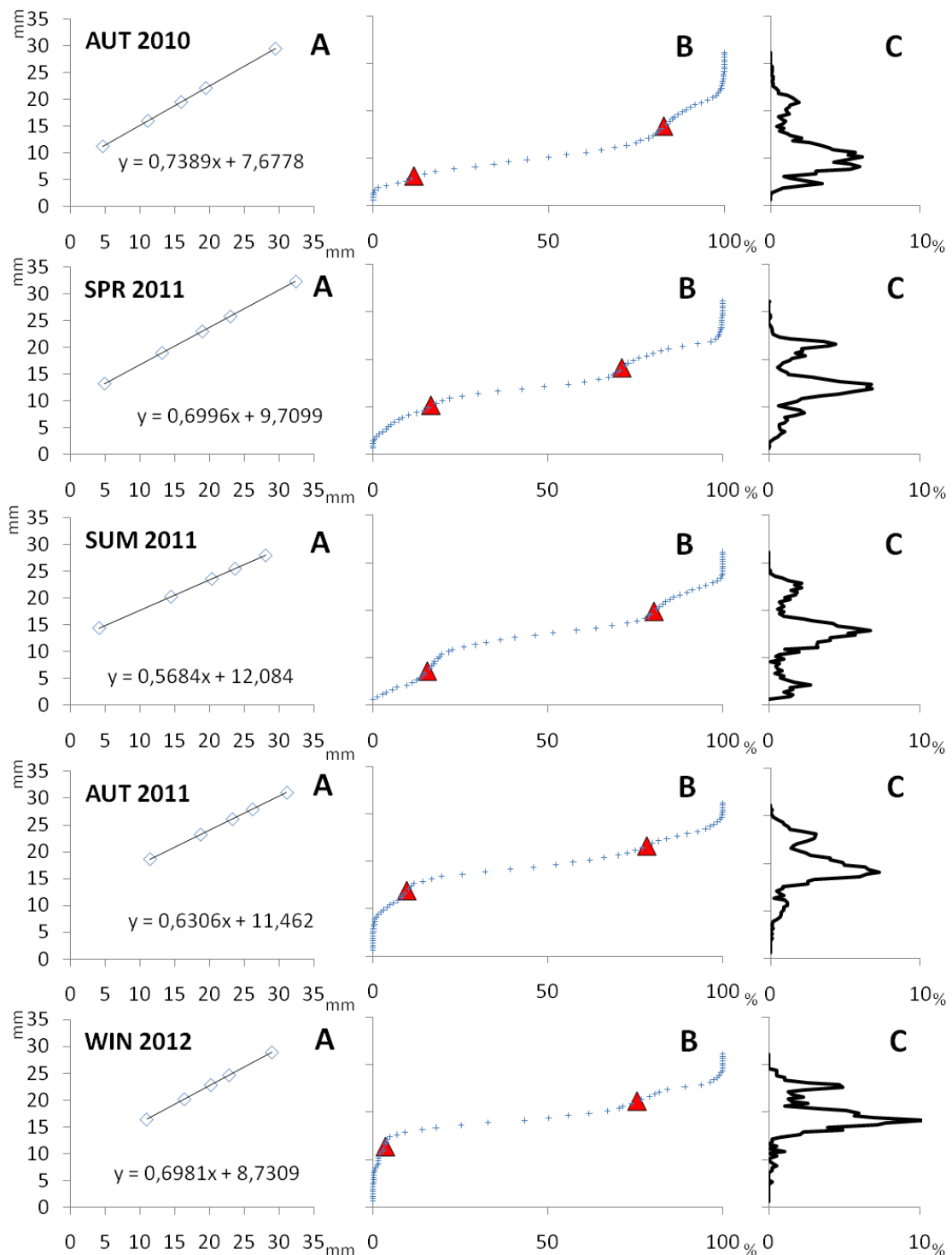
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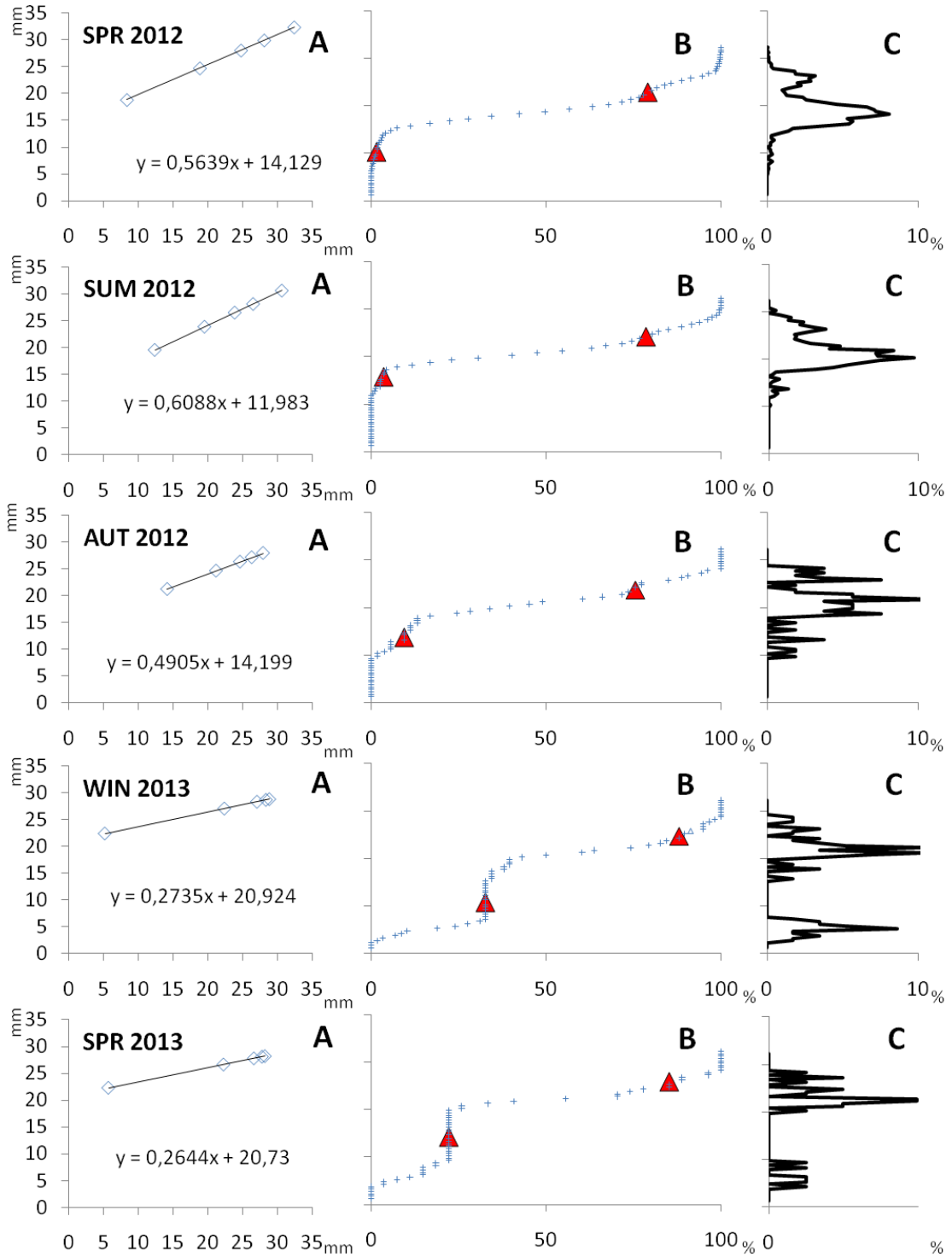
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Appendix 1: Angera - cohorts isolation (from Autumn 2010 to Winter 2012)
 A – Warford method (x-axis = cohort length; y-axis = following cohort length)
 B – Cumulative frequency plots with inflections highlighted in red
 C – Population frequency distributions

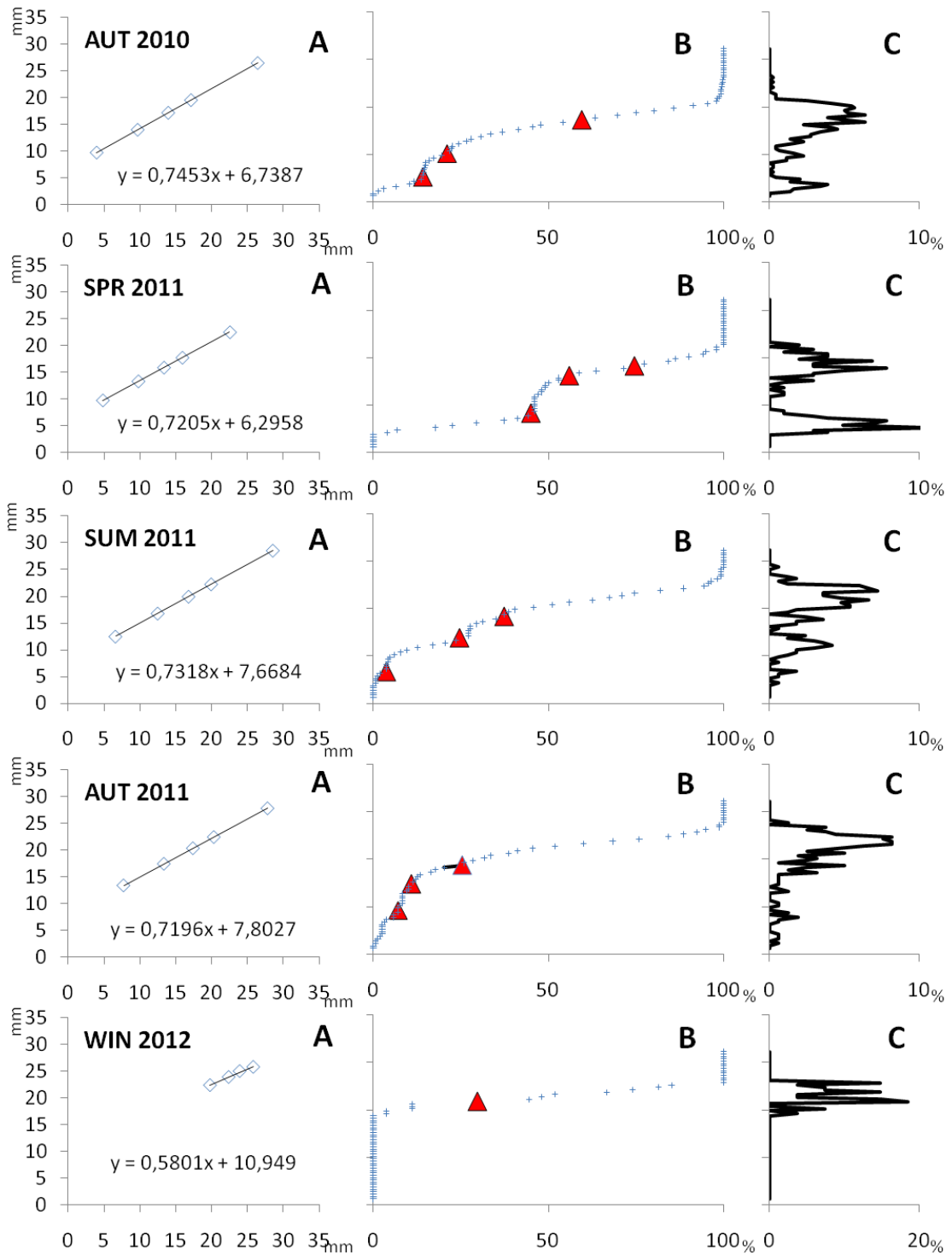


Appendix 2: Angera - cohorts isolation (from Spring 2012 to Spring 2013)

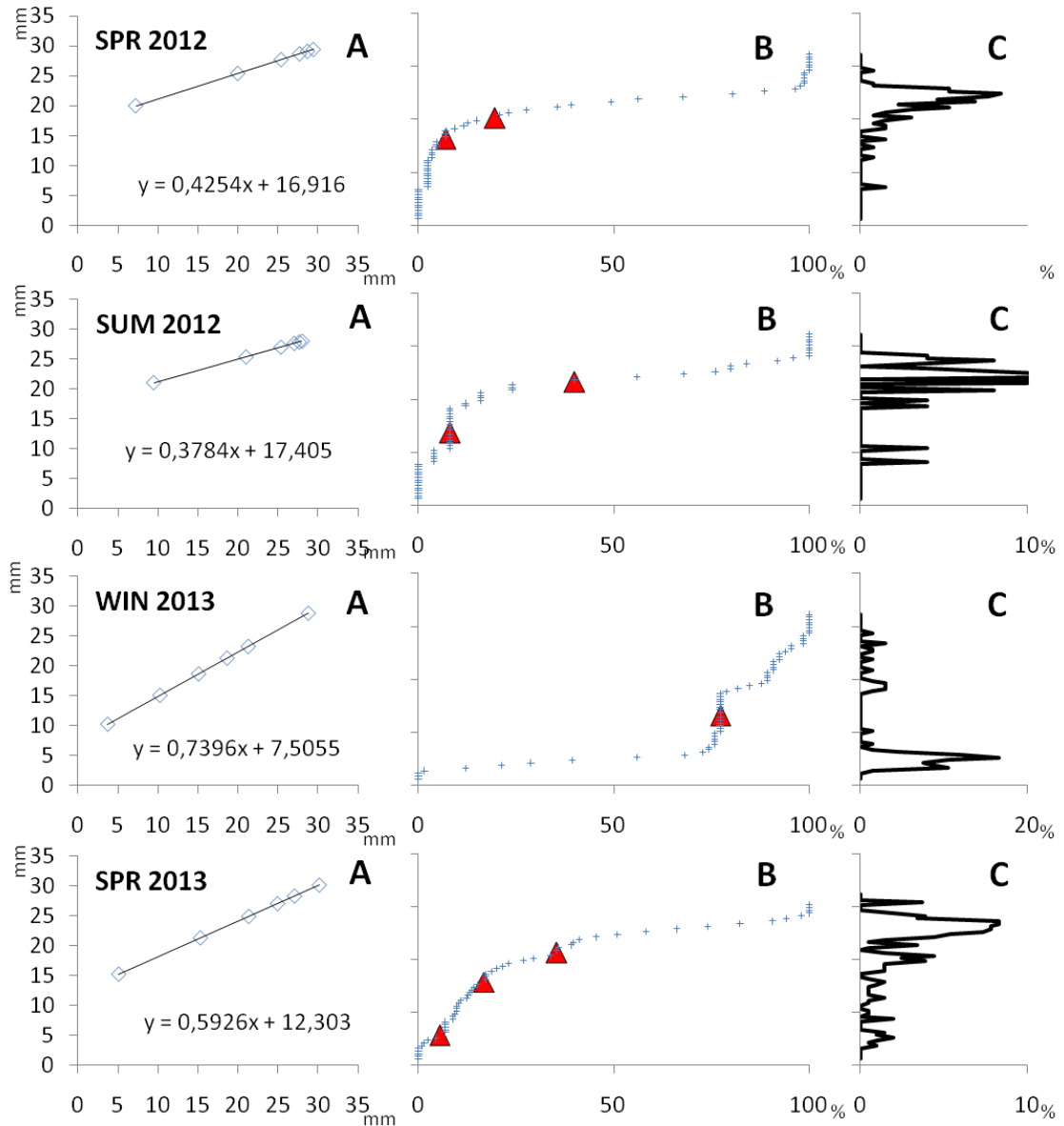
A – Warford method (x-axis = cohort length; y-axis = following cohort length)

B – Cumulative frequency plots with inflections highlighted in red

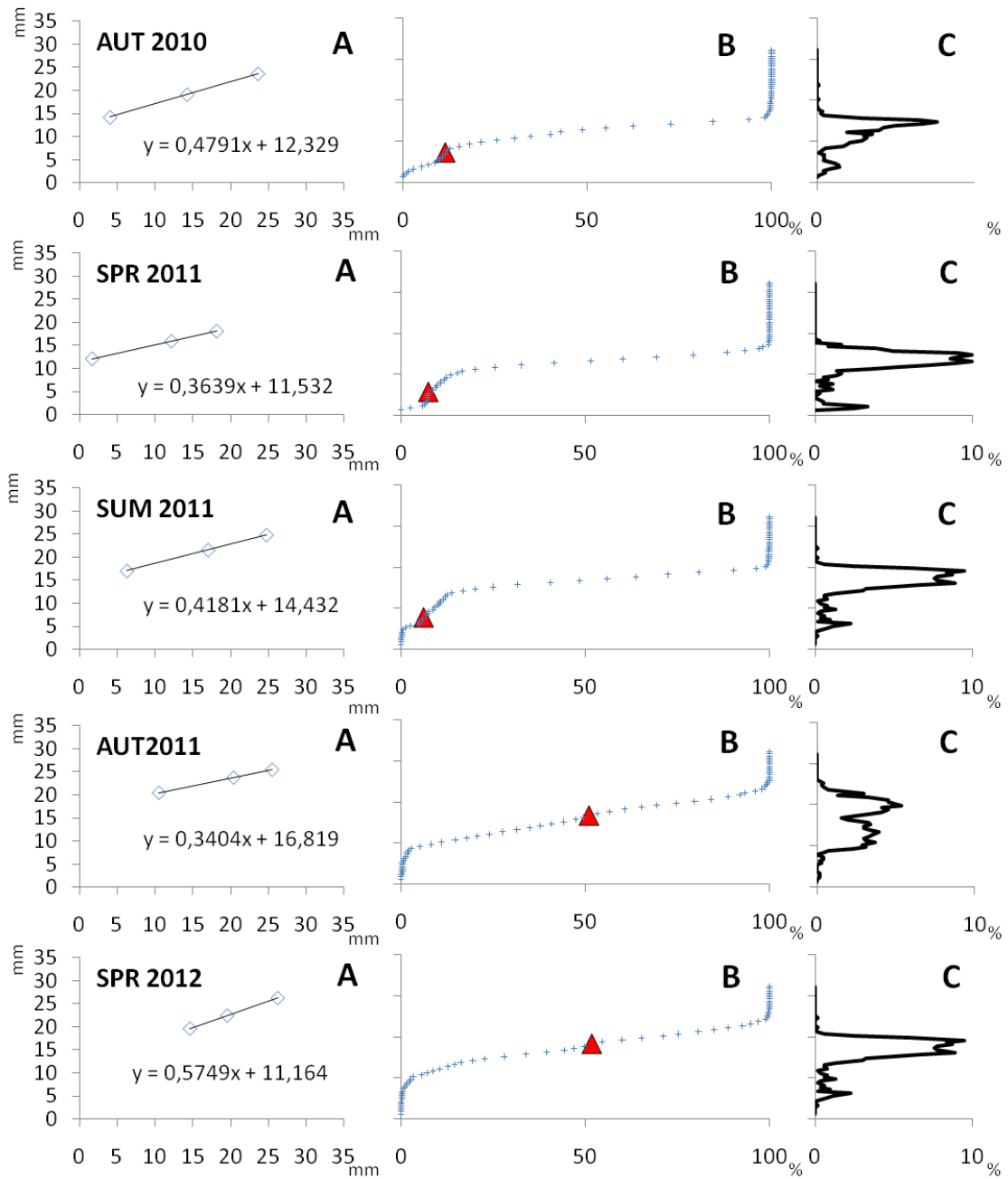
C – Population frequency distributions



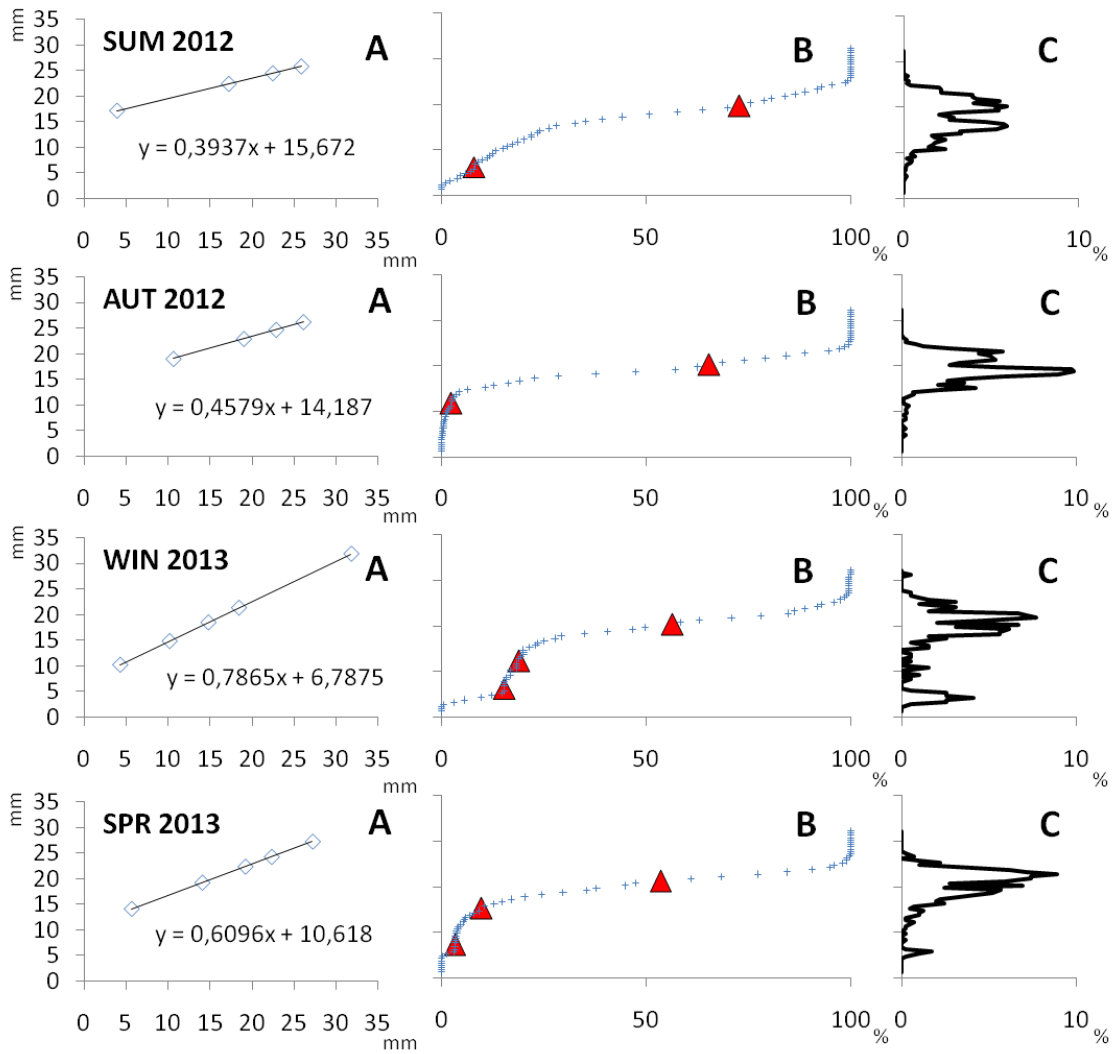
Appendix 3: Arona - cohorts isolation (from Autumn 2010 to Winter 2012)
 A – Warford method (x-axis = cohort length; y-axis = following cohort length)
 B – Cumulative frequency plots with inflections highlighted in red
 C – Population frequency distributions



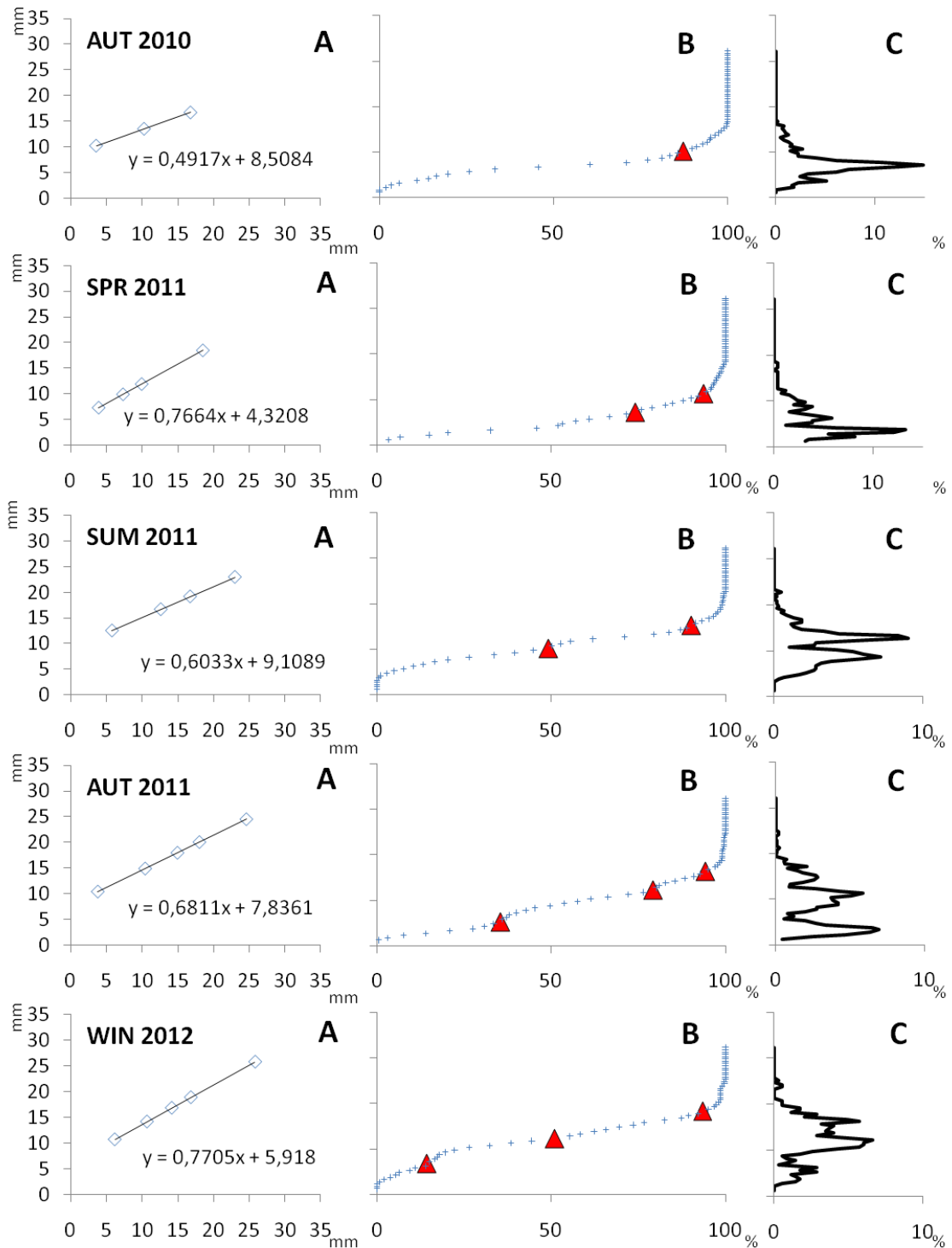
Appendix 4: Arona - cohorts isolation (from Spring 2012 to Spring 2013)
 A – Warford method (x-axis = cohort length; y-axis = following cohort length)
 B – Cumulative frequency plots with inflections highlighted in red
 C – Population frequency distributions



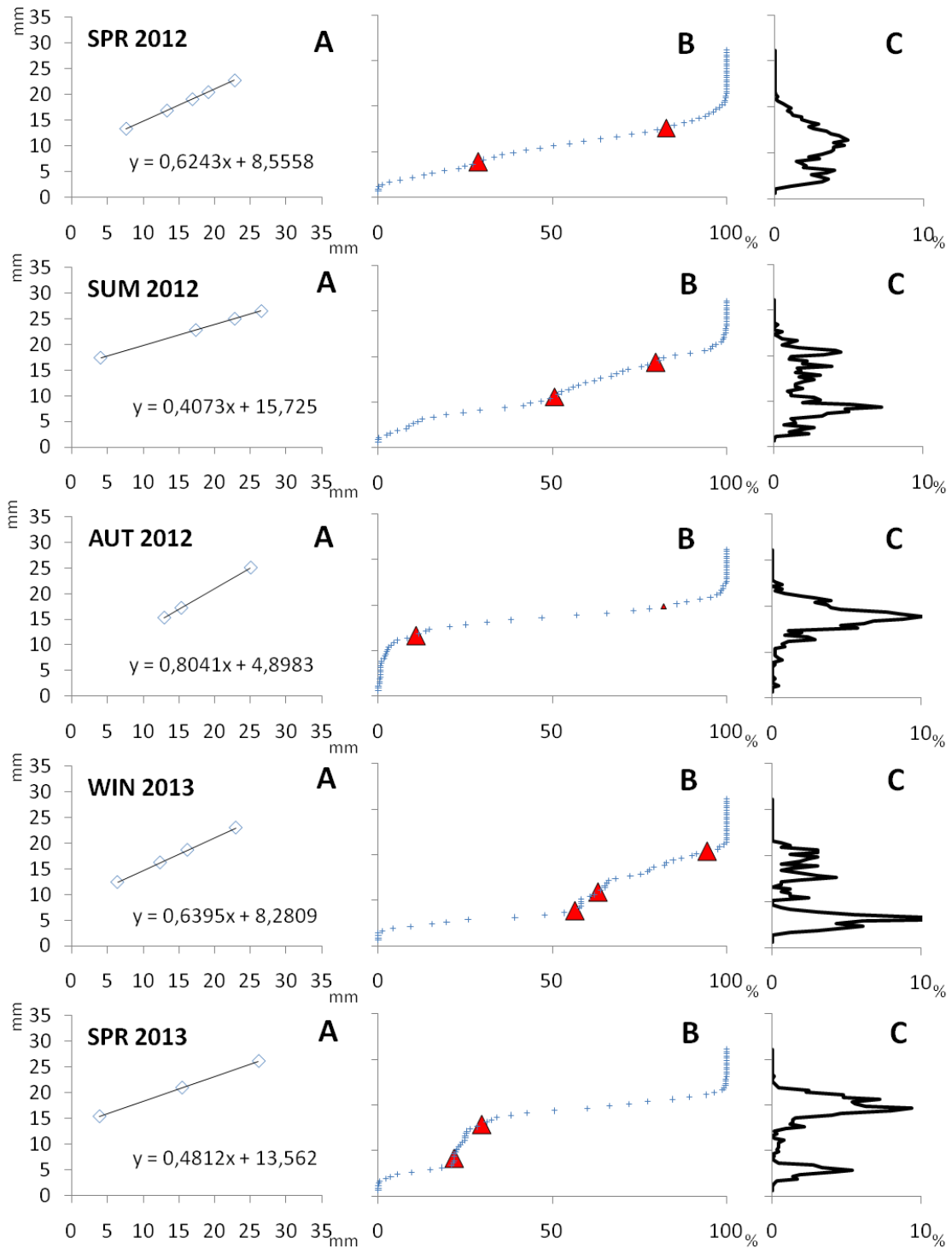
Appendix 5: Brebbia - cohorts isolation (from Autumn 2010 to Spring 2012)
 A – Warford method (x-axis = cohort length; y-axis = following cohort length)
 B – Cumulative frequency plots with inflections highlighted in red
 C – Population frequency distributions



Appendix 6: Brebbia - cohorts isolation (from Summer 2012 to Spring 2013)
 A – Warford method (x-axis = cohort length; y-axis = following cohort length)
 B – Cumulative frequency plots with inflections highlighted in red
 C – Population frequency distributions



Appendix 7: Feriolo - cohorts isolation (from Autumn 2010 to Winter 2012)
 A – Warford method (x-axis = cohort length; y-axis = following cohort length)
 B – Cumulative frequency plots with inflections highlighted in red
 C – Population frequency distributions

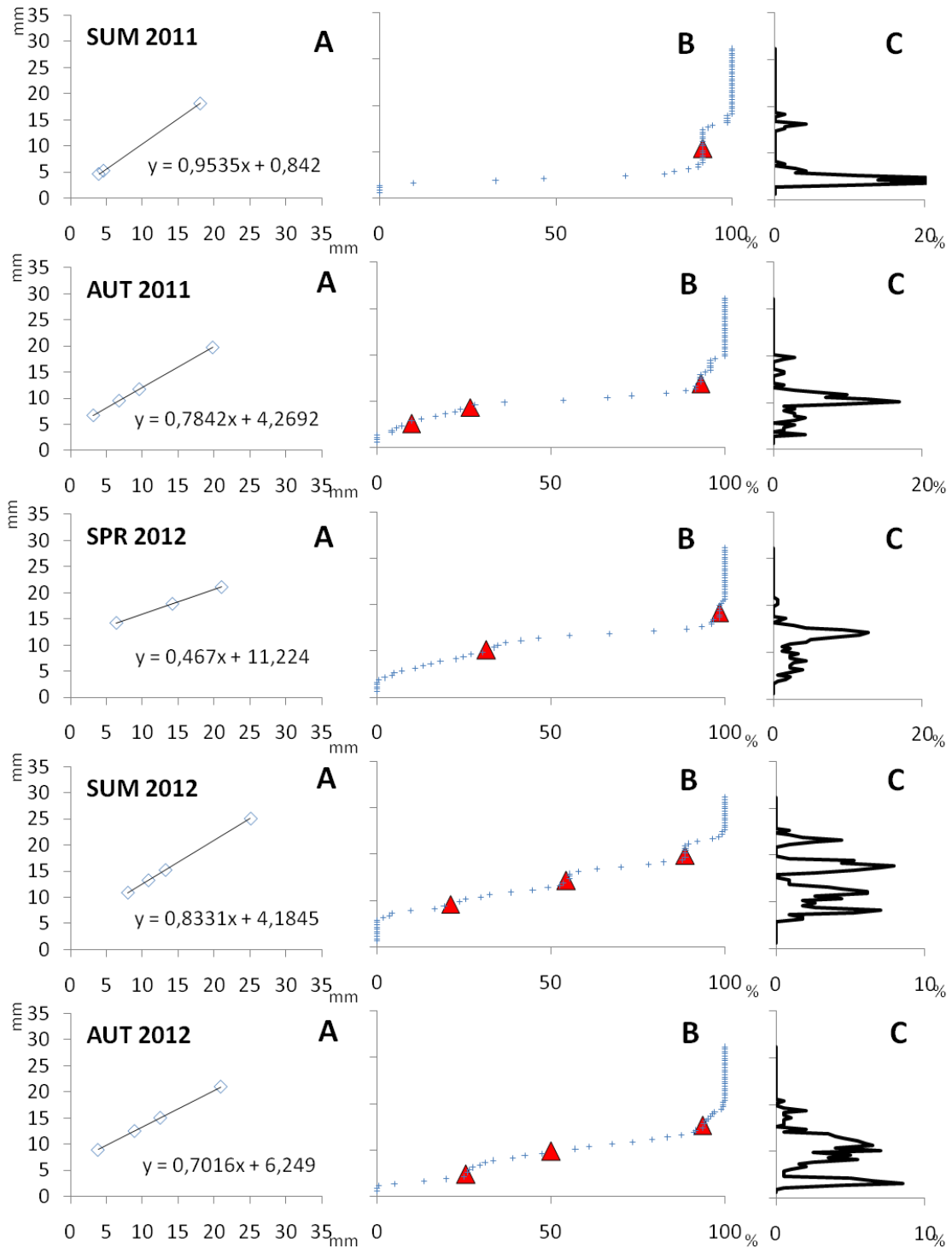


Appendix 8: Feriolo - cohorts isolation (from Spring 2012 to Spring 2013)

A – Warford method (x-axis = cohort length; y-axis = following cohort length)

B – Cumulative frequency plots with inflections highlighted in red

C – Population frequency distributions

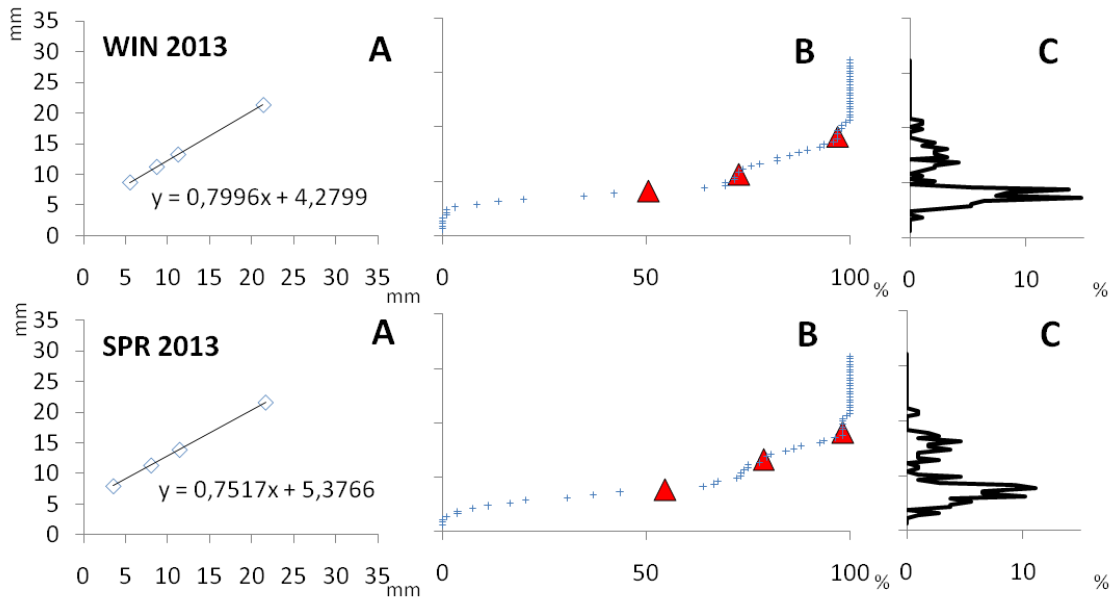


Appendix 9: Monville - cohorts isolation (from Summer 2011 to Autumn 2012)

A – Warford method (x-axis = cohort length; y-axis = following cohort length)

B – Cumulative frequency plots with inflections highlighted in red

C – Population frequency distributions



Appendix 10: Monvalle - cohorts isolation (Winter 2013 – Spring 2013)

A – Warford method (x-axis = cohort length; y-axis = following cohort length)

B – Cumulative frequency plots with inflections highlighted in red

C – Population frequency distributions