JOURNAL OF CRUSTACEAN BIOLOGY, 28(4): 633–640, 2008

THE INVASIVE CRAYFISH *ORCONECTES LIMOSUS* IN LAKE VARESE: ESTIMATING ABUNDANCE AND POPULATION SIZE STRUCTURE IN THE CONTEXT OF HABITAT AND METHODOLOGICAL CONSTRAINTS

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ABSTRACT

Invasive alien species (IAS) potentially may alter all levels of the ecological organization of aquatic water bodies. Therefore, in the context of the EU Water Framework Directive, they represent a significant pressure that should be considered in the assessment of the ecological status of a water body and in the formation of restorative programs. A study was carried out to examine different sampling techniques and to assess the current population structure and the differences in spatial distribution of the alien crayfish *Orconectes limosus* at four sites in Lake Varese (Northern Italy). Three methods were used to assess the crayfish population: mark-recapture, catch per unit effort during night-time snorkelling and quadrat sampling. For the mark-recapture exercise the ventral somites of crayfish were marked using visible implant elastomer tags. A laboratory experiment was used to verify the effectiveness of elastomer tags for the species prior to their use in the field. Crayfish were more abundant at sites characterized by stone substrate than at sites dominated by macrophytes. Hydromorphological alteration of the shoreline (quantified using the newly developed lake habitat survey methodology) at one site appeared to promote the establishment of alien crayfish. Crayfish caught by snorkeling in open water led to an underestimation of the smallest size-classes compared with underwater quadrats. Underwater quadrats on stone substrates provide a quantitative estimation of abundance and size-structure allowing the comparisons of crayfish populations from different lakes. This method could be applied to assess the extent of pressure resulting from alien crayfish under the Water Framework Directive. Crayfish biomass was found to be 82.9% of other macroinvertebrates indicating their importance in the structure and functioning of the lake ecosystem. The presence of a second alien species of crayfish, *Procambarus clarkii*, was recorded for the first time in the lake.

KEY WORDS: Crayfish population, habitat alteration, invasive species, *Orconectes limosus*, quadrat sampling

INTRODUCTION

In the context of the EU Water Framework Directive (WFD, 2000/60/EC), invasive alien species (IAS) represent a significant biological pressure, since these organisms can alter the biological structure and ecological processes of aquatic systems. The assessment of such biological pressures should therefore be considered within an integrated catchment management strategy alongside other pressures and should receive special attention when deciding whether a water body is representative of reference status (REFCOND, 2003; IMPRESS, 2003). The presence of IAS may lead to ecological degradation resulting in a lower classification of a water body and confound the interpretation of ecological assessment metrics.

Invasive alien species are considered the second biggest threat to biodiversity, after habitat loss (IUCN, 2000). However, for lakes, IAS have been considered the principal agent of biodiversity change, largely owing to extensive intentional introductions (Sala et al., 2000). The intentional or accidental spread of IAS into these areas is due to different socio-economic factors: from trade/transport, to the stock of invertebrates and fish for aquaculture, to introductions for sport-fishing and for biocontrol of unwanted organisms (Welcomme, 1988). The impact of IAS may involve many levels of ecological organization including the genetic level, through hybridization with native species, e.g., *Orconectes propinquus* (Girard, 1852) and *O. rusticus* (Girard, 1852) (Perry et al., 2001). Alteration at the community level can occur through habitat alteration, competition, predation and introduction of diseases as well as at the ecosystem level, through alteration of the fluxes of energy and nutrients (Mack et al., 2000; Gherardi, 2007). The degradation of natural habitats itself promotes alien species establishment and invasiveness (IUCN, 2000), as demonstrated for example, by Aguiar et al. (2007) for river systems.

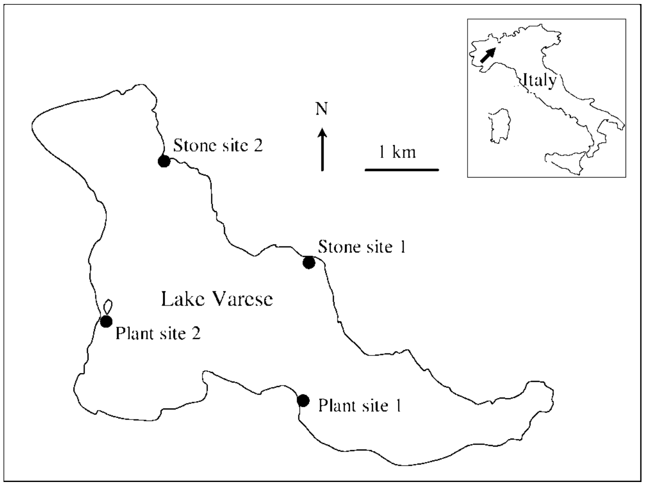

Fig. 1. Map of Lake Varese with sampling sites.

Among freshwater IAS, crayfish are recognized as having substantial potential to alter ecosystems: the UK Environment Agency has listed *Pacifastacus leniusculus* (Dana, 1852) as the second most undesirable species (Evans, 2006). Crayfish are the largest mobile invertebrates inhabiting freshwaters. They are omnivorous, feeding on benthic macroinvertebrates, macrophytes, and detritus and can represent a significant proportion of the benthic biomass (Haertel-Borer et al., 2005). They can represent a significant pressure on the macroinvertebrate community, due to both direct predation (especially on large, less mobile species) and also by causing habitat changes such as the loss of macrophytes (Nyström, 2002; Perry et al., 1997). Even if crayfish can form a large proportion of the diet of some fish species (Haertel-Borer et al., 2005; Momot, 1995), they can have negative impacts on the fish and amphibian communities because they can feed on eggs and compete for food and shelter (Nyström, 2002).

Orconectes limosus (Rafinesque, 1817) is a temperate-water crayfish native to the north-eastern USA (from Maine to Virginia) and south-eastern Canada. It is tolerant to organic-rich waters and general pollutants; although it occurs in rivers, its preferred habitats are ponds and lakes (Hamr, 2002). The first successful introduction into Europe took place in 1890 when Max von dem Borne stocked some specimens from Pennsylvania into a fishpond in Barnowko (north-eastern Germany, now Poland). New introductions in France and successful secondary introductions in many European water bodies, combined with natural dispersal, have resulted in its presence in many European countries (Henttonen and Huner, 1999). It has been recorded in Italy since 1991 (Gherardi et al., 1999) where it was reported in Lake Varese in 2002 (Tartari et al., 2005).

In order to prevent further biological invasions, it is necessary to better understand alien species ecology and to know which factors promote their successful spread and establishment. A study of the crayfish populations in Lake Varese was carried out with the objectives: 1) to estimate the population abundance using a mark-recapture exercise with prior experimental verification of the use of elastomer tags for the species under laboratory conditions, 2) to examine the differences in densities among different habitat types, 3) to determine whether modification of the hydromorphological features of shoreline habitats may promote higher densities, 4) to examine the biomass of crayfish relative to other macroinvertebrates to infer their relative importance in the lake ecosystem, and 5) to propose a simple sampling method to allow among lake comparisons useful in the interpretation of crayfish as a biological pressure, following an evaluation of two methods: quadrat sampling and open-water snorkelling.

Material and Methods

Study Area

Lake Varese, located in sub-Alpine Northern Italy is 14.8 km^2 in area, has a mean depth of 11 m and is moderately eutrophic (Zaccara et al., 2007; Tartari et al., 2005). Four sites were sampled (Fig. 1), two dominated by macrophytes (plant site 1 and plant site 2) and two with stone substrate (stone site 1 and stone site 2).

Characterization of Physicochemistry, Habitat, Macrophyte, and Macroinvertebrate Communities

A habitat survey was carried out at each site following the Lake Habitat Survey (Rowan et al., 2004). The extent of 18 potential pressures was recorded: commercial activities, residential areas, roads or railways, parks and gardens, docks or boats, walls or revetments, recreational beaches, educational recreation, litter or dump, quarrying or mining, coniferous plantation, pasture, tilled land, orchard, pipes or outfalls, dredging, riparian vegetation control, and macrophyte cutting. The extent of these pressures was expressed as an index by summation of scores of either 1 or 0.5 that were awarded if the pressure occurred directly within the site or within a 50 m radius respectively. A score of 0 was assigned if the pressure occurred outside this radius or was absent. The naturalness of the riparian zone was assessed using three components: complexity (count of presence of up to 8 natural structural vegetation types), longevity (presence of $>$10% cover of canopy trees $>$0.3 m in diameter) and naturalness of land cover (cover was assessed as being subject to intensive anthropogenic management or as near-natural). Each component was equally weighted and the sum was expressed on a scale from 0 to 1.

Aquatic macrophytes were sampled during the summer (early September 2006), when vegetative growth is typically at a maximum, along a transect perpendicular to the shoreline at intervals of 0, 2.5, 5, 7.5, 10, 25, 50, 75 and 100 m. Four samples were collected at each site using a double-headed rake grapnel thrown from a boat. The presence of species was recorded from each sample. Results were expressed as percentage of occurrence for each transect. Emergent macrophytes at the lake shore were visually assessed using a 5 point scale of abundance following Palmer (1992).

Macroinvertebrates were collected during spring using a 152 mm^2 Ponar grab. Samples were transferred to a 250 µm mesh net and filtered in situ to remove excess sediment. The remaining sample was preserved in 70% ethanol and subsequently sorted and identified in a laboratory. Wet biomass was measured using an analytical balance (0.0001 g) or estimated from morphometric measurements.

At each site water and sediment samples were taken along with measurements of Secchi depth and sub-surface temperature and oxygen. Alkalinity was measured by titration with H_2SO_4 following Mackereth et al. (1989). Anions and cations were determined by ion chromatography (EPA, 1993; APAT-CNR-IRSA, 2004). Sediment texture was determined for particles smaller in diameter than 2 mm. The sediment texture definitions are given in accordance with the soil texture triangle (Soil Survey Division Staff, 1993). The percentage of the different sediment fractions was determined using graduated sieves and subsequent separation in water (ISO 11277, 1998). Loss on ignition was measured following Heiri et al. (2001).

Crayfish Survey

Information on crayfish population density was estimated in three ways: catch per unit effort, quadrat sampling and multiple mark-recapture. Stone site 2 was selected to compare the three methods owing to easier access to this site. During a preliminary survey, 132 individuals were caught by hand picking and 9 biometrics were measured to the nearest 0.01 mm using a digital callipers: total body length, abdomen length, cephalothorax length (from the tip of the rostrum to the posterior median edge of the cephalothorax), length from the tip of the rostrum to the cervical groove along the cephalothorax, cephalothorax width, abdomen width, chela length, chela width and chela height (following Szaniawska et al., 2005). The wet weight of crayfish was measured using a digital balance (0.001 g). Owing to the high inter-correlations between the biometrics (Pearson's $r > 0.90$), a reduced set of biometrics were recorded for other crayfish sampled: cephalothorax length from the tip of the rostrum to the posterior median edge of the cephalothorax (CL), chela length (CHL; if the chelae were of different sizes the longest one was measured), sex and wet weight. A G-test was used to compare the size class frequencies between captured males and females using R version 2.5.0 following the size-age classification of Pieplow (1938). Differences in cephalothorax length and weight between sexes were tested using a Student's *t*-test. ANCOVA analysis was performed to test for differences in weight and chelae length between the sexes with cephalothorax length as a covariate using Data Desk version 6.1.

An estimate of the relative abundance of crayfish at each site was obtained by hand picking during two hours of night-time snorkelling on 7, 11, 12 and 26 June 2007. Results were expressed per hour as catch per unit effort (CPUE). Crayfish relative abundance at the four sites was compared using a G-test. Quantitative data on crayfish density on stone substrate (stone site 2) was gathered by quadrat sampling at night (4 July 2007). A diver collected all crayfish occurring in 10 underwater quadrats (0.96 m^2 each). Quadrats were set between 0.2 and 1 m depth and consisted of a cylindrical net suspended by a buoyant plastic ring and fixed to the substratum by a metal ring flanked by chain to form a seal on uneven substrates. For each crayfish caught the location of the quadrat, biometrics and wet weight were recorded.

The mark-recapture exercise consisted of four catching sessions at stone site 2 (15 and 30 May 2007; 11 and 25 June 2007). Crayfish were caught during night-time snorkelling and biometrics and wet weight were recorded. The Chapman modification of the Peterson formula was used to estimate the population size at the site (Byrne et al., 1999):

$$N = \frac{(m+1)(c+1)}{r+1} - 1 \qquad (1)$$

where N is the estimated number crayfish in the population, m is the number of marked crayfish available for recapture, c is the number of crayfish in the sample and r is the number of recaptured crayfish in the sample.

Crayfish were individually marked using visible implant elastomer (V.I.E.) tags (Northwest Marine Technology). V.I.E. tags consist of a two-part biocompatible coloured liquid mixture. When injected into abdominal musculature it hardens into a solid mass. Combinations of differently coloured tags implanted in different locations allow a large amount of animals to be individually marked. The potential of this tagging method to persist after the moulting process was tested in a laboratory experiment following the approach of Bubb et al. (2002). Twenty *O. limosus*, ten males and ten females (CL: 22.56-43.24 mm) were caught in Lake Varese and kept in individual tanks that were renewed with de-chlorinated tap water every 6-8 days. Crayfish were fed with slices of carrot and potato and protein supplements (protein 40%). After 2 months of acclimatization, 5 females and 5 males were marked at two positions in the second and one position in the third ventral somite. Tag loss, moult, mortality and presence of eggs were recorded.

Table 1. Habitat characterization, physicochemical characteristics (sampled 2/4/2007), macrophyte composition (submerged and floating: % occurrence in samples, emergent as either dominant: D, abundant: A, frequent: F, occasional: O, or rare: R) following Palmer (1992).

	Plant site 1	Plant site 2	Stone site 1	Stone site 2
Habitat characterization				
Naturalness of riparian zone	0.917	0.833	0.083	0.875
Habitat pressures	0.0	1.5	4.0	2.5
Physicochemical characteristics				
Secchi depth (m)	2.8	3.2	2.8	3.8
Temperature °C (surface)	14.3	14.2	14.9	20.0
O_2 % (surface)	123	100	123	129
O_2 mg l^{-1} (surface)	12.2	9.9	12.1	9.2
pH	8.53	8.23	8.45	8.23
Conductivity (µS cm^{-1})	300	307	310	307
Alkalinity (meq l^{-1})	2.71	2.74	2.76	2.73
Slope	0.019	0.01	0.106	0.116
Predicted distance (m) to 2 m depth contour	72	84	12	7
% Loss on Ignition 550°C	1.45	10.36	1.80	1.00
% Loss on Ignition 950°C	2.25	18.96	9.35	4.53
Cl (mg l^{-1})	9.50	9.79	10.22	9.91
NO_2 (mg l^{-1})	<0.02	<0.02	<0.02	<0.02
NO_3-N (mg l^{-1})	0.32	0.38	0.31	0.39
SO_4 (mg l^{-1})	15.73	16.13	14.57	15.88
PO_4-P (µg l^{-1})	<33	49	42	49
Na (mg l^{-1})	4.91	5.00	5.06	4.53
NH_4 (mg l^{-1})	0.08	0.16	0.07	0.11
Mg (mg l^{-1})	7.94	7.92	7.88	8.30
Ca (mg l^{-1})	39.15	39.63	39.06	41.53
K (mg l^{-1})	2.00	1.99	1.96	1.97
Sediment texture	Sandy loam	Loamy sand	Sand	Sand
Macrophytes				
Emergent:				
Phragmites australis	D	D		
Typha angustifolia	O			
Scirpus sp.	F			
Submerged and floating:				
Ceratophyllum demersum	14	3	0	0
Myriophyllum spicatum	3	0	0	0
Najas marina	47	42	0	0
Nelumbo nucifera	0	42	0	0
Nuphar lutea	14	3	0	0
Nymphaea alba	8	0	0	0
Potamogeton crispus	3	0	0	0
Potamogeton spp.	3	0	0	0
Filamentous algae	19	31	19	25

Results

Site Characterization of Physicochemistry, Habitat, Macrophytes, and Macroinvertebrates

Physicochemical characteristics measured in April 2007 showed little variation among the four sites (Table 1). Plant site 1 and plant site 2 had a structured macrophyte community, with the presence of emergent, submerged and floating species. At the two sites characterized by stone substrate only filamentous algae were found. Three sites had a high degree of riparian naturalness in contrast to stone site 1 which had a very low value as a result of several anthropogenic pressures. This site has undergone substantial hydromorphological modification being changed from natural macrophyte cover to a stone substrate by the construction of a wall, stony shore and pier. The macrophyte sites had a broader littoral zone in comparison to the stone sites as indicated by the shoreline slope and the predicted distance to the 2 m depth contour (Table 1). Macroinvertebrate biomass varied from 97 to 210 kg ha^{-1} at the four sites examined (Fig. 2). Chironomidae and Oligochaeta were typically the dominant groups. Except for Dreissena at stone site 1, there was a notable absence of larger macroinvertebrate taxa at all the sampled sites with few or no specimens of Trichoptera, Gastropoda, Isopoda, Amphipoda, and of the Ephemeroptera only small *Caenis* larvae were found.

Experimental Verification of Tagging Method

During the 80 days of the laboratory experiment eight crayfish moulted once and four twice. Tag retention and visibility at the end of the experiment was 100%. Both total mortality (20%) and fecundity (60% of females) were the same between control and marked crayfish.

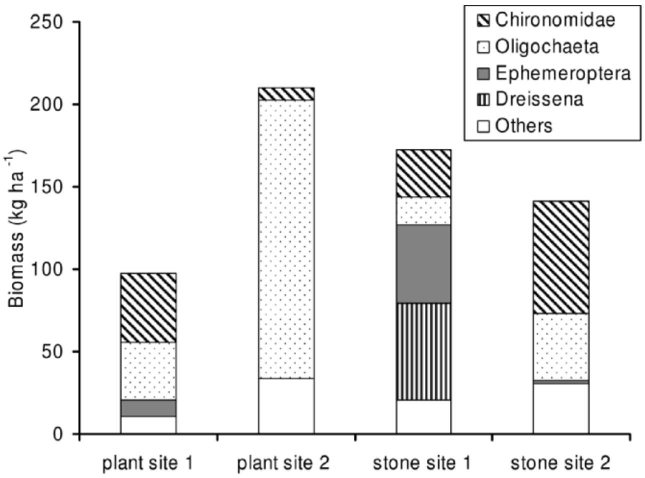

Fig. 2. Wet biomass of macroinvertebrates found at the four sites in Lake Varese.

Biometrics

The total number of *O. limosus* caught during the study period was 673, 46% females and 54% males. The largest male had a carapace length of 50.94 mm, the largest female of 47.26 mm. The mean values of cephalothorax length, chela length, and wet weight are reported in Table 2. No significant difference was found between captured males and females for either cephalothorax length ($t = 0.90$, df = 669, $P > 0.05$) or weight ($t = 1.27$, *d.f.* = 668, $P > 0.05$). However, a significant difference was found between sexes regarding their relative distribution into size classes (G-test: G = 13.0, *d.f.* = 634, $P < 0.01$). An ANCOVA for weight found no evidence for a significant difference between sexes with transformed (x^3) cephalothorax length ($F = 2.15$, $P = 0.15$). As expected, males had longer chelae than females (ANCOVA with transformed ($x^{1.5}$) cephalothorax length: $F = 320$, $P \leq 0.0001$).

Population Size Structure

Figure 3 shows the population size structure (cephalothorax length) as determined by night-time snorkelling at stone site 2 during four sampling occasions. The fitted moving average line indicates a shift in the modal size over the sampling period from 24-27 mm on 15 May to 29-30 mm on 25 June, when evidence is also seen of new recruitment of juveniles.

Crayfish Distribution and Density

Catch per unit effort results (Table 3) indicated that *O. limosus* abundance differed among the 4 sites (G-test with Williams' correction: G = 37.0, *d.f.* = 3, $P < 0.01$), it was highest on natural stone shoreline (stone site 2) and lowest at the two sites dominated by macrophytes. Stone site 1 which has been hydromorphologically modified from macrophyte cover had crayfish abundances up to three times higher than intact macrophyte sites. *Procambarus clarkii* (Girard, 1852), another alien species of crayfish was recorded for the first time in the lake occurring at much lower densities than *O. limosus*.

Table 2. Mean (± standard deviation) of biometrics recorded for female, male, and all crayfish caught during the study. Cephalothorax length: CL, chela length: CHL.

	CL (mm)	CHL (mm)	Wet weight (g)
f (n = 312)	26.87 ± 6.48	14.75 ± 4.58	4.89 ± 3.79
m (n = 361)	27.34 ± 7.05	17.89 ± 6.52	5.28 ± 4.09
tot (n = 673)	27.12 ± 6.79	16.44 ± 5.91	5.10 ± 3.96

The multiple mark-recapture exercise, conducted at stone site 2, estimated the density as 16.7 *O. limosus* m^{-2} with a standard deviation of 7.3. In contrast, a direct estimate of density, conducted using 10 underwater quadrats, estimated the density as 6.4 m^{-2} (SD = 3.9). The average biomass of *O. limosus* found in the quadrats at stone site 2 was 116.90 kg ha^{-1}. In addition to the large difference found in density estimates, there was also substantial difference in the population size structure as determined by the open-water snorkeling method used for mark-recapture and that of quadrat sampling (G-test with Williams' correction for comparison of size class frequencies between the two methods: G = 50.5, *d.f.* = 3, $P < 0.01$). Figure 4 shows that snorkeling tended to grossly underestimate the smaller (<1 yr) individuals which were estimated to form just 19% of the population as compared to 77% using quadrats.

Discussion

Crayfish were 3 to 8 times more abundant on stone habitats than plant habitats as determined by catch per unit effort for the four sites sampled. This was most likely a result of stone habitats providing refuges from predation and cannibalistic behaviour (Capelli and Magnuson, 1983). This finding is coherent with that of Lodge and Hill (1994) who found crayfish abundance to increase with the percentage of littoral zone occupied by cobble substrate.

Modification of the shoreline at one site (stone site 1) from macrophyte cover to an artificial stone shoreline likely promoted the establishment of crayfish which were three times more abundant than at sites with natural macrophyte cover. Also *Procambarus clarkii*, a new introduction to the lake occurred at highest abundance there. Site 1 also contained the invasive bivalve *Dreissena polymorpha* (Fig. 2), and its presence was likely to have been facilitated by the availability of suitable solid substrates such as boulders and walls (Mackie, 1991). Anthropogenic habitat disturbance as an agent promoting the abundance and number of alien species has also been observed in other biological groups such as stream macrophytes (Aguiar et al., 2007). Both habitat destruction and alien species introductions are pressures that likely act in synergy and are key causes of biodiversity loss (Sala et al., 2000), a fact also acknowledged in the recent EU communication aimed at stopping biodiversity loss (European Community, 2006).

The newly developed lake habitat survey (Rowan et al., 2004; Rowan et al., 2006) was successful in detecting habitat modification (Table 1) and such data, gathered as part of EU member states implementation of the Water Framework Directive (2000/60/EC), may be useful for incorporation into risk assessments for alien species colonisation and success.

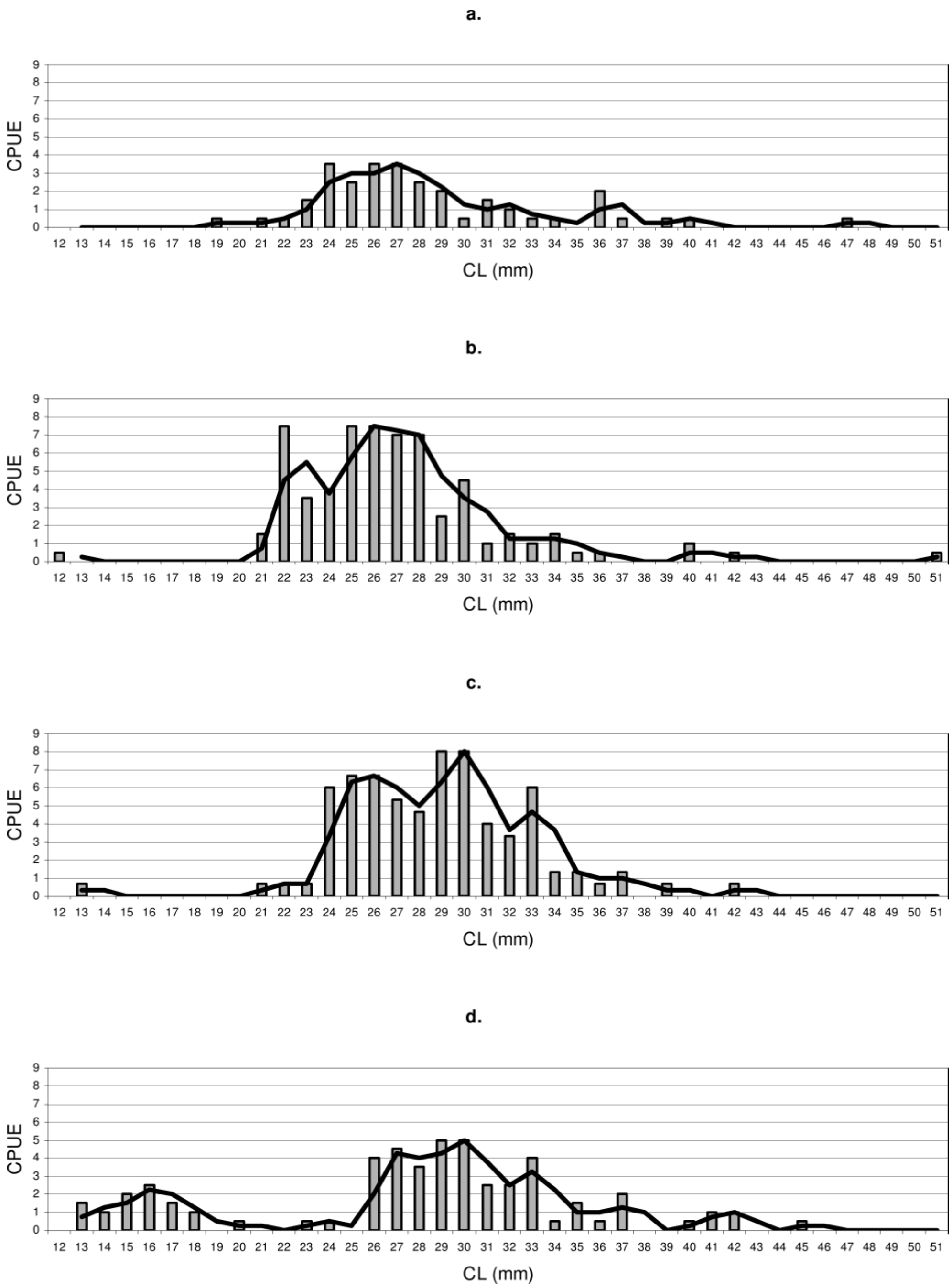

Fig. 3. Population size structure as indicated by cephalothorax length (CL) over four sampling occasions at stone site 2 with fitted moving average. Sampling dates: a. 15th of May; b. 30th of May; c. 11th of June; d. 25th of June 2007.

Crayfish have the potential to act as keystone species having a pivotal role in determining ecosystem structure and functioning (Favreau et al., 2006; Momot, 1995). Experimental work on *P. clarkii* has indicated that it can strongly affect communities of macroinvertebrates and macrophytes even at low densities of 4 m^{-2} (Gherardi and Acquistapace, 2007). Estimates of density (stone site 2) of *O. limosus* in this study ranged from 16.7 to 6.4 ind. m^{-2} based on mark-recapture and quadrat sampling respectively, so a substantial impact on littoral communities would be expected. The average biomass (stone site 2) of *O. limosus* determined using quadrats was 116.90 kg ha^{-1} which was 82.9% that of other macroinvertebrates inferring their importance as keystone species. The complete absence of submerged and floating macrophytes from the stone sites (Table 1) could be a result of the high crayfish densities which can lead to increased consumption or mechanical damage of vegetation (Momot, 1995; Gherardi and Acquistapace, 2007). The high occurrence of filamentous algae at all sites sampled (Table 1) may be a result of the eutrophic status of Lake

Table 3. Abundance (catch per unit effort, CPUE) of *O. limosus* and *P. clarkii* at four sites expressed per hour of night-time snorkelling.

Crayfish	Plant site 1	Plant site 2	Stone site 1	Stone site 2
CPUE *O. limosus*	8	11	33	67.33
CPUE *P. clarkii*	0.5	0	1	0.13

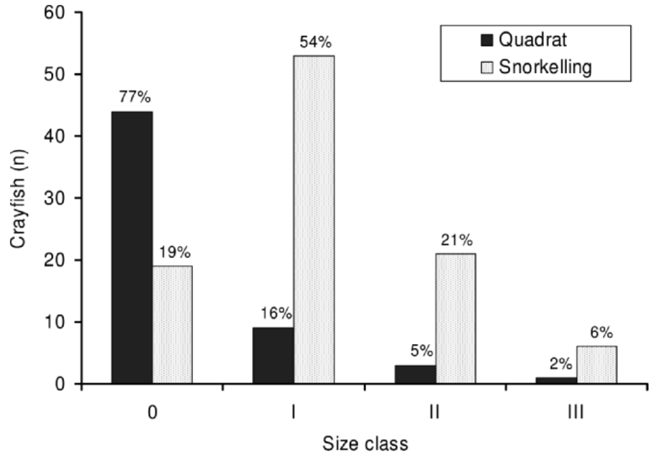

Fig. 4. Size-age classes (after Pieplow, 1938) using two different sampling methods. The percentage that each size class comprised of total catch is expressed separately for snorkelling and quadrat sampling.

Varese (Zaccara et al., 2007; Tartari et al., 2005). One contributing factor may however be that crayfish tend to selectively prey on grazers such as snails only found at one site in this study, which has been experimentally associated with an increase in periphyton biomass (Nyström et al., 1999). This may in turn further restrict macrophyte growth through epiphytes coating their surface thereby reducing light availability (Phillips et al., 1978). Crayfish have also been found to have a significant effect on surface microalgae biomass associated with direct grazing (Gherardi and Lazzara, 2006). Therefore the presence of alien crayfish may cause a cascading top-down effect that may alter the whole ecosystem and lead to a progressive reduction of biodiversity (Gherardi, 2006; Gherardi, 2007). A further insight into the role of alien crayfish in the food web of lake Varese could be achieved by incorporating information on predation rates by fish and carrying out stable isotopes analysis on key components of the littoral community (Nyström et al., 1999; Haertel-Borer et al., 2005). The occurrence of *Procambarus clarkii* at much lower densities than *O. limosus* may indicate that it is still in an early stage of colonising the lake.

In estimating population abundance through a mark-recapture technique, a prerequisite is the knowledge about how the marking procedure may affect the probability of recapture. The experimental study on the use of visible implant elastomer tags for *O. limosus* indicated that it was clearly visible through the ventral somites, was retained after moulting and allowed for individual identification. Therefore visible implant elastomer tags can be considered an effective tagging method for *O. limosus*. It should be preferred to other marking methods for crustaceans since it allows long-term individual identification and the tagging of even small size-classes. In contrast, other tags such as external marks are lost during moults (Gherardi et al., 2000) and internal transponders (Bubb et al., 2002) and streamer tags (Kimker et al., 1996) have minimum size limitations. These tags have also been used successfully for other crustacean species. For example, Clark and Kershner (2006) found that tag retention and tag visibility in both adult and juvenile *Orconectes obscurus* (Hagen, 1870) were 100%; Frisch and Hobbs (2006) found a tag retention of 98% over a long-term period in a wild population of *Panulirus versicolor* (Latreille, 1804).

Methodological differences in sampling techniques present a barrier to comparing biometric data acquired for *O. limosus* with previous studies. For example, Szaniawska et al. (2005) reported much larger values of mean cephalothorax length: 45.3 mm for females and 46.7 mm for males in the Vistula lagoon (Poland). These values are over 68% higher than those found in Lake Varese (cf. Table 2) probably because fyke-nets were used to catch crayfish. Fyke-nets, like traps, selectively sample bigger and more active crayfish (Westman and Pursiainen, 1982; Byrne et al., 1999) while night-time snorkeling and especially quadrat sampling allow smaller individuals to be caught.

No significant difference was found in weight between males and females for either captured specimens as a whole or through analysis of covariance with cephalothorax length. This contrasts with the findings of Ďuriš et al. (2006) whose data-set contained a greater proportion of larger individuals (caught from brooks) and indicated that growth curves differed with sex but only for larger specimens (>60-65 mm total length).

Substantial difference was found in the population age structure as determined by snorkeling and quadrat sampling. The catch during snorkeling tended to underestimate the smallest age-class. This is most likely a result of larger crayfish being more visible and sampled preferentially. In contrast, quadrat sampling serves to focus sampling intensively over a small area. Quadrat sampling also provides a direct estimate of crayfish density; compared to this method, mark-recapture data may overestimate population density owing to lake shores being open systems where unknown mortality, migration and immigration make estimates difficult. Lamontagne and Rasmussen (1993) found quadrats to be a more precise method of sampling crayfish than timed counts, while Hein et al. (2006) reported them to be less effective on soft substrate where crayfish may escape before the quadrat is placed on the substrate. The less biased determination of size structure provided by quadrats would be beneficial for modeling the alteration of lake ecosystems. This is because juvenile and adult crayfish are considered separate food web components, the former playing an important role as prey for fish and birds while the latter have a stronger influence on communities of macrophytes and snails (Gherardi, 2007).

Across the EU, monitoring programs to determine the ecological quality of lakes were initiated towards the end of 2006 as required by legislation (WFD, 2000/60/EC). However guidance on how to deal with alien species as a 'biological pressure' affecting ecological quality is currently lacking. Given the interrelationships of alien species of crayfish with many of the biological groups required to be monitored (fish, macroinvertebrates, macrophytes, and phytobenthos) their incorporation into assessment systems is essential. Underwater quadrats may provide quantitative data with a less biased estimation of size structure allowing comparisons with other biological groups and among lakes when similar habitats are sampled. However, there is a need for more fundamental research into how functional interrelationships are altered by alien crayfish at ecosystem scale (Haertel-Borer et al., 2005).

Acknowledgements

We thank Bruno Paracchini and Maria Vito of the Institute for Environment and Sustainability, Joint Research Centre for technical assistance. We thank the editors of this journal and two anonymous reviewers for comments that helped the revision of this manuscript.

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RECEIVED: 5 December 2007.
ACCEPTED: 30 January 2008.