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Can biotic resistance be utilized to reduce establishment rates of non-indigenous species in constructed waters?

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Abstract Understanding the mechanisms that facilitate establishment of non-indigenous species is imperative for devising techniques to reduce invasion rates. Passively dispersing non-indigenous organisms, including zooplankton, seemingly invade constructed waters (e.g., ornamental ponds, dams and reservoirs) at faster rates than natural lakes. A common attribute of these invaded water bodies is their relatively young age, leading to the assertion that low biotic resistance may lead to their higher vulnerability. Our aim was to determine if seeding of young water bodies with sediments containing diapausing stages of native zooplankton could accelerate community development, leading to greater biotic resistance to the establishment of new species. Twenty outdoor tanks were filled with water (1,400 L) and nutrients added to attain eutrophic conditions. Ten treatment tanks had sediments added, sourced from local water bodies. In the remaining ten, sediments were autoclaved, and received zooplankton via natural dispersal only. In an initial 12 month monitoring period, species richness increased at a greater rate in the treatment tanks (at 12 months average standing richness per tank = 3.8, accumulated richness = 8.2) than control tanks (2.6 and 5.0, $P < 0.05$). Treatment tanks developed assemblages with greater proportions of species adapted to pelagic conditions, such as planktonic cladocerans and copepods, while control tanks generally comprised of smaller, littoral dwelling, rotifers. Analysis of similarities indicated community composition differed between the control and treatment groups at 12 months ($P < 0.01$). Two copepod, four rotifer and one cladoceran species were intentionally added to tanks at 12 months. In the 3 month post-introduction period, five of these species established populations in the control tanks, while only two species established in the treatment tanks. The calanoid copepod *Skistodiaptomus pallidus*, for example, a non-indigenous species confined to constructed waters in New Zealand, established exclusively in tanks where native calanoid copepod species were absent (primarily control tanks). Our study suggests that biotic resistance could play an important role in reducing the establishment rate of non-indigenous zooplankton. It also provides evidence that seeding constructed water bodies with sediments containing diapausing eggs of native species may provide an effective management tool to reduce establishment rates of non-indigenous zooplankton.

Keywords Exotic species · Dams · Reservoirs · Managerial tool · Zooplankton

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Introduction

Non-indigenous species are a major threat to biodiversity, and it is therefore important to elucidate patterns in invasions that can be used to reduce their establishment rates. One emerging trend in aquatic systems is that non-indigenous organisms, particularly passively dispersing taxa such as zooplankton, plants and algae, commonly invade constructed lakes (e.g., reservoirs for water supply and electricity generation, ornamental ponds, retired mine pits and quarries) at faster rates than natural lakes, which subsequently act as hubs for spread to natural systems (e.g., Havel et al. 2005; Johnson et al. 2008; Banks and Duggan 2009). One of the first recognised examples of invasion of non-indigenous zooplankton occurring preferentially in constructed waterways was that of the cladoceran *Daphnia lumholtzi*, native to the old world tropics, which invaded a reservoir in Texas, USA, in 1990. In one decade it had spread through at least 125 lakes in North America, with reservoirs typically at the invasion front (Havel et al. 1995). Similarly, the Australasian calanoid copepod *Boeckella triarticulata* was first recorded in northern Italy in constructed fish ponds in the 1980s, and has subsequently spread to adjacent natural water bodies and constructed lakes in southern-Italy (Ferrari and Rossetti 2006; Alfonso and Belmonte 2008). More broadly, an extensive study conducted by Johnson et al. (2008), which explored data from 1080 water bodies in Wisconsin and Michigan, USA, suggested five high profile invaders, Eurasian watermilfoil (*Myriophyllum spicatum*), zebra mussel (*Dreissena polymorpha*), rusty crayfish (*Orconectes rusticus*), spiny waterflea (*Bythoytrephes longimanus*) and rainbow smelt (*Osmerus mordax*), were all more likely to occur in constructed than natural lakes. New Zealand has similar examples, including the Australian calanoid copepods *Boeckella minuta* and *B. symmetrica*, the Japanese *Sinodiaptomus valkanovi* and North American *Skistodiaptomus pallidus*, all of which have to date been recorded only in constructed waters (Duggan et al. 2006; Banks and Duggan 2009; Makino et al. 2010). Havel et al. (2005) identified several characteristics of reservoirs that may potentially increase their vulnerability to invasion relative to natural lakes. These characteristics include reduced biotic resistance due to their young age, high physical disturbance and greater environmental variability. In contrast to the North American studies, however, Banks and Duggan (2009) found that the higher frequency of non-indigenous zooplankton occurrences in New Zealand constructed waters was not confined to dams, but extended to ornamental ponds, disused mine pits, quarries and farm dams. These water bodies are more similar to natural waters than dams with respect to connectivity and disturbance, leading to the suggestion by these authors that biotic resistance is a more compelling factor.

The concept of biotic resistance suggests that better developed communities with, for example, greater species richness, are more resistant to invasion than those with fewer species (Elton 1958). Experimental trials have found, for instance, that high plant diversity in a recipient area exerts a negative effect on establishment success of introduced species (e.g., Tilman 1997; Kennedy et al. 2002). Although derived primarily from terrestrial ecosystems, biotic resistance has also been demonstrated experimentally for zooplankton in constructed ponds (Shurin 2000; Dzialowski 2010). However, the importance of biotic resistance has also recently been contested; the role of propagule pressure—the number and/or the frequency of propagules released at introduction—has been recognised as highly important, with all systems apparently susceptible to invasion if a sufficient propagule supply is available (e.g., von Holle and Simberloff 2005). Nevertheless, if biotic resistance is important in reducing invasion rates in aquatic systems, it follows that introducing native propagules into new habitats at an early stage of reservoir filling may accelerate community development, and thus provide biotic resistance to invasion during a potentially vulnerable time.

Zooplankton can take a number of years to develop mature communities in new aquatic habitats (e.g., Ejsmont-Karabin 1995), and are slow to colonise by natural means (i.e., by wind, rain or waterfowl). For example, few zooplankton species are typically found to establish in short-term experimental colonisation studies (i.e., one to two years; Jenkins and Buikema 1998; Cáceres and Soluk 2002). Constructed waters, in their initial stages of development, may thus have highly undeveloped communities, and be relatively open for invasion. Opportunities for establishment of non-indigenous species facilitated by human mediated vectors (e.g., in association with fish introductions, with sediment carried on construction equipment, dumping of aquaria) may therefore be very important for species establishment during these formative years. "Seeding" of new aquatic habitats with native propagules to increase the rate of development of zooplankton communities has not been tested, but may provide a potential tool to reduce species invasion rates, provided propagule supplies of non-indigenous species are not overwhelming.

The overall aim of this study was to determine experimentally whether "seeding" of young water bodies, with sediments containing native zooplankton eggs, will increase the rate of community development, and provide an effective tool to reduce the establishment rate and spread of non-indigenous species.

Methods

Tank setup and monitoring

To examine if seeding water bodies with native propagules accelerates zooplankton community development, leading to greater biotic resistance against the subsequent introduction of non-indigenous species, 20 experimental tanks were set up for a period of 15 months. Each tank had a maximum capacity of 1,800 L (dimensions $\sim$ L = 1.5 m $\times$ W = 1.0 m $\times$ H = 1.2 m). Tanks were filled with 1,400 L of tap water, leaving 30 cm between the top of the tank and the surface of the water. Space was left between tanks to allow access during monitoring and to minimize the chance of cross-contamination from splashing during rainfall. Nitrogen and phosphorus (7.46 g of NH_3Cl and 6.41 g of K_2PO_4) were added to attain eutrophic conditions similar to local water bodies (equivalent to concentrations of 1.44 mg/L N and 0.82 mg/L P). Other micronutrients were provided using a synthetic pond water formulation modified from Hebert and Crease (1980; 119 g $NaHCO_3$, 94.24 g $CaSO_4$ and 74.4 g $MgSO_4$ into 1,400 L). Water was left for one day before introductions of sediment to reduce any effects of chlorine on zooplankton and/or their eggs.

Tanks were randomly separated into ten control tanks and ten treatment tanks. On 7 September 2008 each tank had a total of 300 g of surface sediments added, sourced from three shallow local habitats containing diapausing eggs representative of local zooplankton communities (Lake Ngaroto, an unnamed farm pond in Gordonton, and Chapel Lake at the University of Waikato). Sediments were obtained at a depth of approximately 1 m from each water body, and each contributed 100 g of sediment to the total introduced. Sediments released into control tanks were autoclaved so that diapausing eggs contained within the sediments were killed. Control tanks represented new water bodies colonised by zooplankton via natural vectors, while treatment tanks represented new water bodies seeded with natural lake sediments. The same volume and origin of sediments were used for both the control and treatment ponds to negate any differences in community composition potentially arising from, for example; (1) differences in chemicals (including nutrients) arising from the sediments, (2) contrasting habitats for potential benthic species that may prey on zooplankton eggs, or (3) habitat variability caused by sediments providing a refuge for zooplankton species. Communities were left to colonise by natural means for 12 months. During this period no zooplankton or algae (food) were intentionally added to the tanks.

Monitoring began one day after sediments were added. Subsequent monitoring took place twice monthly thereafter for a total of 15 months. Zooplankton monitoring was carried out using a 9 cm diameter PVC integrated plankton cylinder with a total height of 1.12 m. The cylinder was vertically submerged into the water to a depth of 74.5 cm, approximately 15 cm above the bottom of the tank, capped, and lifted from the tank, sampling 4.74 L of water. Two cylinder samples were obtained per tank on each monitoring day and combined into a single sample of 9.48 L. The collected water was filtered through a 40 μm sieve over the tank, so that unfiltered water was returned to the tank. Zooplankton retained on the filter was washed into a sample container and preserved in ethanol.

Temperature, conductivity and specific conductance were measured using a YSI 30M, and oxygen concentration and saturation using a YSI 550A meter. Measurements were taken approximately 15 cm below the surface and 15 cm from the bottom of each tank, so that two readings were obtained per tank for each environmental variable. Chlorophyll *a* was collected as a 200 mL surface water (15 cm depth) sample and measured using a calibrated fluorometer following acetone extraction. All equipment was washed thoroughly with tap water between tanks to avoid cross contamination during sampling. After 8 months of monitoring, one control tank acquired an unfixable leak and was excluded from the remaining experiment. Ten treatment and nine control tanks were thus used for the remaining monitoring period.

After a 12 month colonisation period (7 September 2008 to 7 September 2009), seven new zooplankton taxa not found in any of the tanks during the initial 12 months were intentionally introduced into the tanks over a one week period. These were the rotifer *Synchaeta* spp (40 individuals per tank, a mix of *S. pectinata* and *S. oblonga*), the cyclopoid copepod *Mesocyclops leuckarti* s.l. (25 individuals), calanoid copepod *Skistodiaptomus pallidus* (16 individuals), cladoceran *Chydorus* sp. (5 individuals), and the rotifers *Ascomorpha ovalis* (4 individuals) and *Trichocerca similis* (3 individuals). Species were added at a variety of abundances as, if we had introduced each in the same numbers, all may have established in every tank, or none will have established in any tank. Introducing species at a variety of abundances thus increased the probability of achieving introduction levels where some species might be more likely to establish in the unseeded tanks, and be less likely in the seeded tanks (i.e., at levels where propagule pressure and biotic resistance interact). Care was taken to ensure new individuals were alive at the time of introduction. One treatment and one control tank did not receive any new species after 12 months; this was done to elucidate whether any of the new species introduced at 12 months might also have become established through natural means during this time. As one control tank was lost from the experiment, a total of eight control tanks and nine treatment tanks had additional species added at 12 months. A subsequent 3 month period was used to monitor the colonisation success following new species introduction.

Zooplankton were enumerated using a dissecting microscope at c. 30× magnification, and identified with a compound microscope using standard identification guides. As unidentifiable copepod nauplii larvae were first to establish in most tanks, these were counted as a species in diversity estimates following their first appearance; the next identifiable copepod species established in any tank was thus not counted as an additional new species.

Statistical analysis

Environmental variables and species richness for treatment and control groups at the time new species were added (12 months) were compared with *t* tests using STATISTICA (Statsoft Inc., Tulsa. USA). Analysis of similarities (ANOSIM) was performed to determine if differences existed in community composition between treatment and control groups at 12 months, using the PRIMER v6 statistical package (Clarke and Gorley 2006). ANOSIM is a non-parametric permutation test that provides a measure of the dissimilarity of groups of samples in the form of an *R*-statistic. *R* values closer to zero indicate that groups are similar, while values approaching one indicate groups are very dissimilar. ANOSIM was applied to a Bray-Curtis similarity matrix calculated from log $(x + 1)$ transformed abundance data to down weigh the contribution of highly abundant species, using treatment and control tanks as groups.

Results

Environmental variables

Temperatures were similar between treatment and control tanks throughout the 15 month monitoring period. A maximum of 26.1°C was recorded in early January 2009 (austral summer; Fig. 1a), and a minimum of 4.9°C in early June 2009 (austral winter). Dissolved oxygen concentrations (DO) and saturation (%O_2) followed similar patterns (Fig. 1b, c). Major differences were not observed between treatment and control tanks. Average DO and %O_2 reached their maxima in October 2008. Minimum DO was recorded in December 2008, and lowest average %O_2 was obtained in April 2009. After initial variability, pH remained fairly stable (Fig. 1d). Minimum pH levels were recorded following tank setup (7.4), and were greatest in October 2008, 1.5 months after the experiment started (8.9). There were no major differences in pH between treatment and control tanks at any time, with the greatest average difference of 0.6 recorded in June 2009. Specific conductance (@ 25°C) remained similar between the treatment and control groups (Fig. 1e). Average specific conductance was greatest (453.1 μS cm^{-1}) at the beginning of monitoring in September 2008, falling to a minimum in October 2009

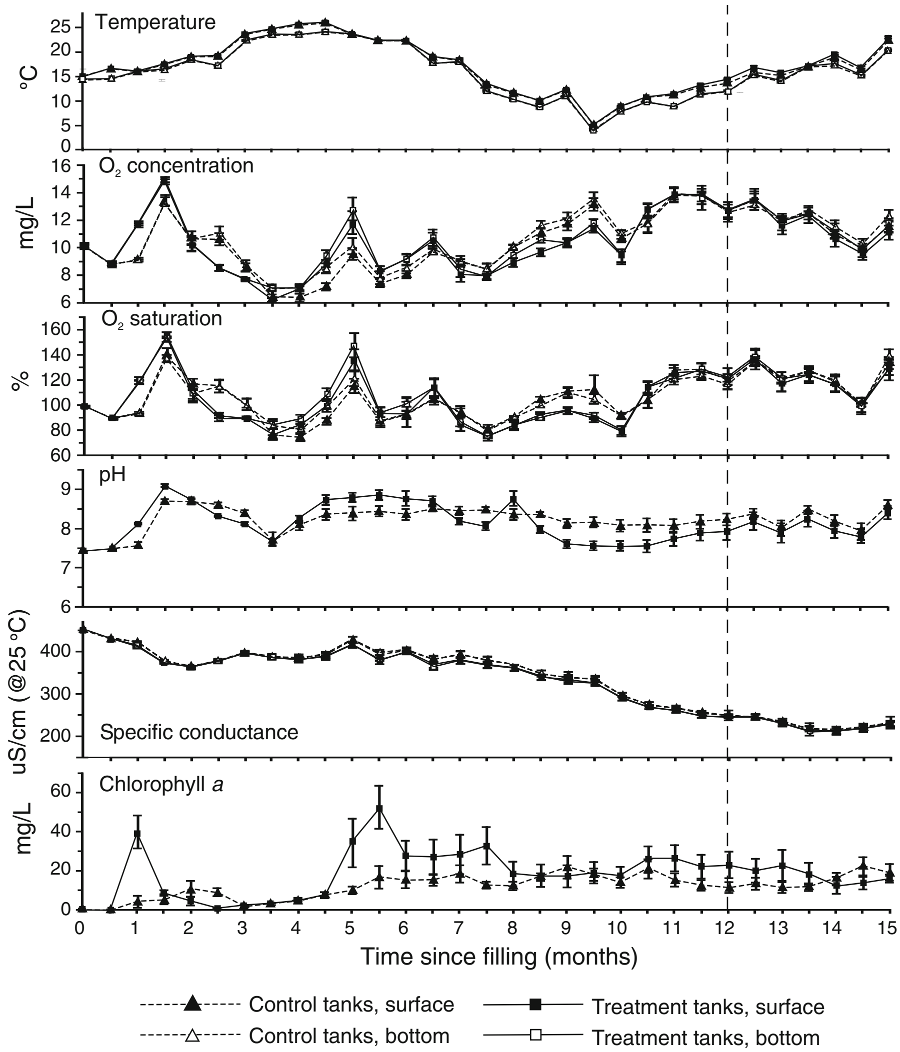

Fig. 1 Environmental conditions in the treatment and control tanks throughout the 15 month monitoring period. The *dashed vertical line* indicates time of species introduction. *Error bars* indicate ±1 standard error of the mean

(212.1 μS cm^{-1}). Chlorophyll *a* varied between treatment and control tanks (Fig. 1f). Control tanks had minimum concentrations of 0.04 mg L^{-1} immediately following tank set up, and a maximum of 22.38 mg L^{-1} in November 2009 (14.5 months after set up). Treatment tanks exhibited greater variation, and chlorophyll *a* levels were generally higher than in control tanks. The lowest chlorophyll *a* concentration in treatment tanks was 0.06 mg L^{-1} at the start of the experiment, with a maximum of 52.64 mg L^{-1} in early February 2009 (5.5 months after commencement). When the new species were introduced at 12 months, there were no statistically significant differences for any environmental variable between control and treatment groups (all $P > 0.05$; Table 1). These results infer that, at the time new zooplankton species were introduced, similar environmental conditions occurred in the treatment and control tanks.

Zooplankton species richness

Species richness in general increased gradually over the 15 month monitoring period in both control and treatment tanks, but at a higher rate in the treatment tanks (Fig. 2). Control tanks did not begin to markedly increase until approximately 5 months following tank set-up, rising to a mean standing richness of 2.6 species at 12 months. In contrast, species richness in treatment tanks began to increase immediately following set up, attaining an average of 3.8 species at 12 months. Accumulated mean species richness (i.e., the total number of species recorded in

Table 1 Mean values of environmental variables in treatment and control tanks at 12 months, and *P* value indicating statistical significance

Environmental variable	Treatment	Control	*P* value
Temperature, surface (°C)	13.24 (0.80)	12.80 (0.60)	0.092
Temperature, bottom (°C)	11.54 (0.50)	11.37 (0.45)	0.113
Specific conductance, surface (μS cm^{-1})	249.33 (28.84)	255.44 (12.31)	0.378
Specific conductance, bottom (μS cm^{-1})	249.22 (16.64)	255.65 (13.01)	0.364
DO concentration, surface (mg L^{-1})	12.52 (1.95)	12.55 (0.96)	0.972
DO concentration, bottom (mg L^{-1})	12.71 (1.97)	12.54 (1.95)	0.821
DO saturation, surface (%)	127.23 (20.90)	128.97 (10.06)	0.831
DO saturation, bottom (%)	126.67 (19.53)	124.57 (9.03)	0.79
pH	8.19 (0.68)	8.38 (0.44)	0.457
Chlorophyll *a* concentration	23.11 (21.53)	11.48 (8.52)	0.145

Standard deviations are given in brackets

tanks up until that time) was also observed to increase at a greater rate in the treatment tanks than the control tanks, attaining average richness' of 8.2 and 5.0 species at 12 months, respectively. Standing mean species richness in treatment and control tanks was significantly different at this time (*t* test $P = 0.0017$), as was accumulated mean diversity ($P = 0.0001$).

Following the introduction of new species at 12 months, average species richness in the control tanks increased rapidly. Within 1.5 months of the introductions, average mean standing richness in the control tanks rose from 2.6 to 4.3, as the new species established. In contrast, relatively little change was observed in the standing or accumulated species richness' of the treatment tanks throughout the same period (an average total increase of 0.8 species). Between 12 and 15 months the accumulated richness in control tanks increased on average by 2.4 species, relative to 0.7 species in the treatment tanks. At 15 months, neither the mean standing nor accumulated richness' between treatment and control tanks were statistically different ($P > 0.05$).

Zooplankton community composition

In the treatment tanks, copepod nauplii were observed in one tank from day 1, with the copepod *Acanthocyclops robustus* s.l. established after 1 month, and *Lepadella ovalis*, a rotifer, present by 1.5 months (Table 2). After 2.5 months three cladocerans were also present, *Moina tenuicornis*, *Ceriodaphnia dubia* and *Pleuroxus hastirostris*, along with the copepod *Boeckella delicata* and ostracod *Eucypris virens*. The majority of the rotifer species, *Trichocerca pusilla*, *Squatinella mutica*, bdelloids and *Cephalodella catellina*, did not establish until at least 5 months after the experiment began. Fifteen different species were observed in the treatment tanks through the initial 12 months. The unseeded control tanks took substantially longer to accumulate species over the initial 12 month monitoring period (Table 3). Of the ten taxa recorded during the first 12 months, seven (*Lepadella ovalis*, *Trichocerca pusilla*, *Lecane flexilis*, *Squatinella mutica*, *Filinia longiseta*, Bdelloids and *Cephalodella catellina*) were small rotifers. The remaining species, two cladocerans (*C. dubia* and *P. hastirostris*) and one cyclopoid copepod (*A. robustus* s.l.), were all found in the treatment tanks at an earlier stage.

The community composition at 12 months in the treatment tanks differed greatly to that of the control tanks (Table 4). At 12 months, eleven species (*Acanthocyclops robustus* s.l., *Lepadella ovalis*, *Squatinella mutica*, *Boeckella delicata*, *Calamoecia lucasi*, *Eucypris virens*, *Filinia longiseta*, bdelliods, *Cephalodella catellina*, *Moina tenuicornis* and *Ceriodaphnia dubia*) were present within the treatment tanks. Five of these are truly planktonic copepods or cladocerans, another five were rotifers, and one was an ostracod. Seven species (*A. robustus* s.l., *L. ovalis*, *Lecane flexilis*, *S. mutica*, bdelloids, *C. catellina* and *C. dubia*) were present in the unseeded control tanks. All but two of these species, the cyclopoid copepod *A. robustus* s.l. and cladoceran *C. dubia*, were

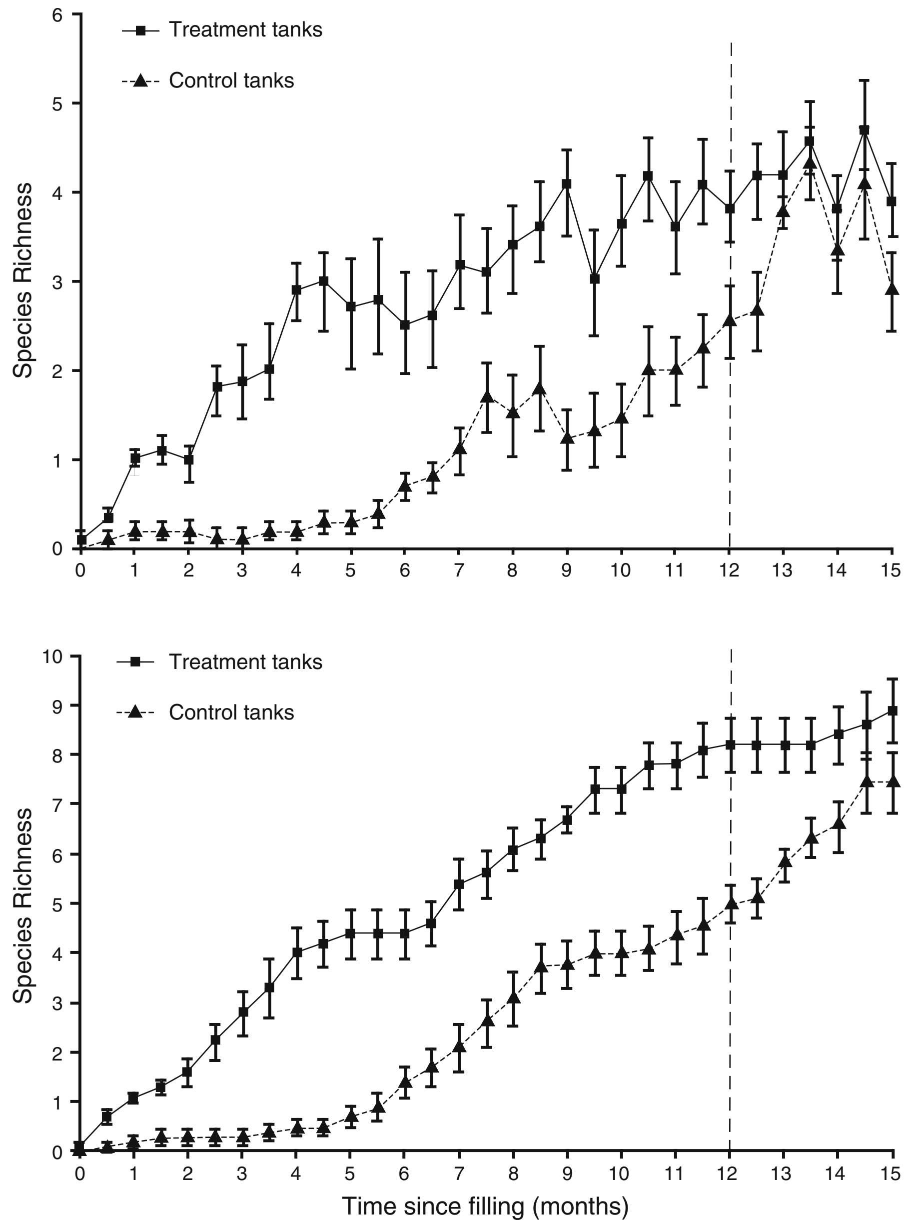

Fig. 2 Standing mean species richness (*above*) and accumulated richness (*below*) in treatment and control tanks. The *dashed vertical line* indicates time of species introduction. *Error bars* indicate ±1 standard error of the mean

rotifers. Results of the ANOSIM analysis indicated that community composition differed significantly between the treatment and control tanks at 12 months ($R = 0.503$, $P < 0.01$).

At 15 months, species composition between treatment and control tanks differed greatly from those present at 12 months. Treatment tanks still had a greater number of species overall, but of the species introduced at 12 months the control tanks had acquired more of the new species and in a greater proportion of tanks (Table 4). Control tanks acquired five new species (*Skistodiaptomus pallidus*, *Synchaeta oblonga*, *Mesocyclops leuckarti* s.l., *Chydorus* sp. and *Synchaeta pectinata*) while treatment tanks gained only two new species (*S. pallidus* and *C. sphaericus*). The two species established in the treatment tanks were only present in four out of the nine treatment tanks into which new species were released, and within these four tanks, only a single species established per tank. In contrast, the new species established in all eight of the control tanks into which they were introduced. Three (38%) of the

Table 2 Species establishment throughout the 15 month monitoring period in treatment tanks (asterisk indicates presence in at least one tank)

Month	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Species											
Copepod nauplii	*	*	*	*	*	*	*	*	*	*	*
Acanthocyclops robustus			*	*	*	*	*	*	*	*	*
Lepadella ovalis				*							
Moina tenuicornis					*	*	*	*	*	*	*
Ceriodaphnia dubia					*	*	*	*	*	*	*
Boeckella delicata						*	*	*	*	*	*
Eucypris virens						*	*	*		*	*
Pleuroxus hastirostris						*	*		*	*	*
Filinia longiseta							*	*	*	*	
Calamoecia lucasi								*	*	*	*
Bdelliods											*
Squatinella mutica											
Trichocerca pusilla											
Lecane flexilis											
Cephalodella catellina											
Bosmina meridionalis											
Daphnia carinata											
Species added at 12 months											
Skistodiaptomus pallidus											
Synchaeta oblonga											
Synchaeta pectinata											
Mesocyclops leuckarti											
Chydorus sp.											
Trichocerca similis											
Ascomorpha ovalis											

Month	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
Species										
Copepod nauplii	*	*	*	*	*	*	*	*	*	*
Acanthocyclops robustus	*	*	*	*	*	*	*	*	*	*
Lepadella ovalis					*	*			*	*
Moina tenuicornis	*	*	*	*	*	*	*	*	*	*
Ceriodaphnia dubia	*	*	*	*	*	*	*	*	*	*
Boeckella delicata	*	*	*	*	*	*	*	*	*	*
Eucypris virens	*	*	*	*	*	*	*	*	*	*
Pleuroxus hastirostris	*	*	*	*					*	
Filinia longiseta	*			*	*		*	*	*	*
Calamoecia lucasi	*	*	*	*	*	*	*	*	*	*
Bdelliods	*			*	*		*	*	*	*
Squatinella mutica								*	*	*
Trichocerca pusilla			*	*	*	*	*	*	*	*
Lecane flexilis										
Cephalodella catellina									*	*

Table 2 continued

Month	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
Bosmina meridionalis										
Daphnia carinata										
Species added at 12 months										
Skistodiaptomus pallidus										
Synchaeta oblonga										
Synchaeta pectinata										
Mesocyclops leuckarti										
Chydorus sp.										
Trichocerca similis										
Ascomorpha ovalis										

Month	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0
Species										
Copepod nauplii	*	*	*	*	*	*	*	*	*	*
Acanthocyclops robustus	*	*	*	*	*		*	*	*	*
Lepadella ovalis	*	*	*	*	*	*	*	*	*	*
Moina tenuicornis	*	*	*	*	*	*	*	*	*	*
Ceriodaphnia dubia	*	*	*	*	*	*	*	*	*	*
Boeckella delicata	*	*	*	*	*	*	*	*	*	*
Eucypris virens	*	*	*	*	*	*	*	*	*	*
Pleuroxus hastirostris					*				*	*
Filinia longiseta	*	*	*	*	*	*	*	*	*	*
Calamoecia lucasi	*	*	*	*	*	*	*	*	*	*
Bdelliods	*	*	*	*	*	*	*	*	*	*
Squatinella mutica		*	*	*	*	*	*	*	*	
Trichocerca pusilla			*				*	*	*	
Lecane flexilis										
Cephalodella catellina	*	*	*	*	*	*	*	*	*	
Bosmina meridionalis			*							*
Daphnia carinata									*	*
Species added at 12 months										
Skistodiaptomus pallidus									*	
Synchaeta oblonga										
Synchaeta pectinata										
Mesocyclops leuckarti										
Chydorus sp.								*	*	
Trichocerca similis										
Ascomorpha ovalis										

New species were introduced at 12 months. Species are ordered by their first appearance in either treatment or control tanks

tanks gained four species, while one other had three species establish (i.e., 50% of tanks had three or more species establish). Two of the new species, the copepods *Skistodiaptomus pallidus* and *Mesocyclops leuckarti* s.l., established in seven of the eight control tanks (88%). In contrast, *S. pallidus* only established in two of nine (22%) treatment tanks that received new species, while *M. leuckarti* did not establish in

Table 3 Species establishment throughout the 15 month monitoring period in control tanks (asterisk indicates presence in at least one tank)

Month	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Species											
Copepod nauplii			*							*	
Acanthocyclops robustus				*	*						
Lepadella ovalis											
Moina tenuicornis											
Ceriodaphnia dubia									*	*	*
Boeckella delicata											
Eucypris virens											
Pleuroxus hastirostris											
Filinia longiseta											
Calamoecia lucasi											
Bdelliods											
Squatinella mutica											
Trichocerca pusilla											
Lecane flexilis											
Cephalodella catellina											
Bosmina meridionalis											
Daphnia carinata											
Species added at 12 months											
Skistodiaptomus pallidus											
Synchaeta oblonga											
Synchaeta pectinata											
Mesocyclops leuckarti											
Chydorus sp.											
Trichocerca similis											
Ascomorpha ovalis											

Month	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
Species										
Copepod nauplii					*		*			
Acanthocyclops robustus										
Lepadella ovalis		*	*	*	*	*	*	*	*	*
Moina tenuicornis										
Ceriodaphnia dubia	*	*	*	*	*	*	*	*	*	*
Boeckella delicata										
Eucypris virens										
Pleuroxus hastirostris					*	*	*	*	*	*
Filinia longiseta										
Calamoecia lucasi										
Bdelliods		*	*	*	*	*	*	*	*	*
Squatinella mutica	*					*				
Trichocerca pusilla			*	*		*	*		*	*
Lecane flexilis									*	*
Cephalodella catellina										

Table 3 continued

Month	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0
Bosmina meridionalis										
Daphnia carinata										
Species added at 12 months										
Skistodiaptomus pallidus										
Synchaeta oblonga										
Synchaeta pectinata										
Mesocyclops leuckarti										
Chydorus sp.										
Trichocerca similis										
Ascomorpha ovalis										

Month	10.5	11.0	11.5	12.0	12.5	13.0	13.5	14.0	14.5	15.0
Species										
Copepod nauplii		*				*	*	*	*	*
Acanthocyclops robustus										
Lepadella ovalis	*	*	*	*	*	*	*	*		*
Moina tenuicornis										
Ceriodaphnia dubia	*	*	*	*	*	*	*	*	*	*
Boeckella delicata										
Eucypris virens										
Pleuroxus hastirostris	*	*	*		*		*		*	
Filinia longiseta		*								
Calamoecia lucasi										
Bdelliods	*		*	*	*	*	*	*	*	*
Squatinella mutica			*	*	*	*	*	*		
Trichocerca pusilla	*				*		*			
Lecane flexilis	*	*	*	*	*	*	*	*	*	
Cephalodella catellina	*		*	*	*	*				
Bosmina meridionalis										
Daphnia carinata										
Species added at 12 months										
Skistodiaptomus pallidus						*	*	*	*	*
Synchaeta oblonga						*	*	*	*	*
Synchaeta pectinata						*	*	*	*	*
Mesocyclops leuckarti								*	*	*
Chydorus sp.									*	*
Trichocerca similis										
Ascomorpha ovalis										

New species were introduced at 12 months. Species are ordered by their first appearance in either treatment or control tanks

any treatment tank. The rotifers *Synchaeta pectinata* and *Synchaeta oblonga* established in three (25%) and two (38%) of control tanks, respectively, while neither established in any of the treatment tanks. Besides *S. pallidus*, the cladoceran *Chydorus* sp. was the only other new species to establish in the treatment tanks. It established in 22% of the treatment tanks and 38% of the control tanks. The rotifers

Table 4 Species present at 12 and 15 months in each treatment and control tank

	Control Tanks									Treatment Tanks									
	1	3	6	7	11	14	15	18	*20*	2	4	5	8	10	12	13	*16*	17	19
Species at 12 months																			
Acanthocyclops robustus s.l.					*									*			*	*	
Lepadella ovalis	*	*	*	*	*	*	*	*	*	*	*				*	*		*	
Lecane flexilis	*																		
Squatinella mutica	*					*									*	*			
Boeckella delicata										*	*	*	*	*			*		*
Calamoecia lucasi										*	*		*				*		*
Eucypris virens										*	*		*	*					*
Filinia longiseta														*					*
Bdelliods		*		*		*							*	*					
Cephalodella catellina		*					*								*	*			
Moina tenuicornis													*						
Ceriodaphnia dubia			*	*			*	*									*	*	*
Species at 15 months																			
Acanthocyclops robustus s.l.												*						*	
Lepadella ovalis	*			*				*	*							*			*
Boeckella delicata										*	*		*	*			*	*	*
Calamoecia lucasi										*	*			*			*	*	*
Eucypris virens											*			*	*				*
Filinia longiseta																		*	*
Bdelloids				*					*							*	*		
Moina tenuicornis													*						
Ceriodaphnia dubia			*	*			*	*					*			*	*	*	*
Pleuroxus hastirostris																	*		
Bosmina meridionalis																*			
Daphnia carinata																		*	
Skistodiaptomus pallidus	*	*		*	*	*	*	*				*			*				
Synchaeta oblonga		*		*				*											
Mesocyclops leuckarti s.l.		*	*	*	*	*	*	*											
Chydorus sp.		*	*			*					*		*						
Synchaeta pectinata				*				*											

Tanks 16 and 20 (italics) did not receive new species at 12 months

Ascomorpha ovalis and *Trichocerca similis* did not establish in any of the tanks during the 3 month period. None of the new species were found to establish in the two tanks that did not receive new species at 12 months (Tanks 16 and 20), indicating establishment likely came about due to our deliberate introductions rather than by other means.

Discussion

Overall, our results indicate that biotic resistance plays an important role in reducing the establishment rates of non-indigenous zooplankton. The results also provide strong evidence that zooplankton community development can be accelerated as a management tool to reduce establishment rates of non-indigenous species. A substantially lower establishment rate of zooplankton was observed in tanks seeded with zooplankton resting eggs. These eggs acted to increase species richness, increase community development rates, and increase biotic resistance towards subsequent invasions. The seeding of new lakes with appropriate species therefore should provide a practical tool for environmental managers to reduce establishment rates of non-indigenous zooplankton in constructed water bodies.

The seeding of tanks with sediments containing diapausing stages of native zooplankton species significantly increased the rate of community development. Species richness in the seeded tanks increased rapidly and community composition began to diverge from control tanks almost immediately following tank set-up. By 3 months, three cladocerans (*Moina tenuicornis, Ceriodaphnia dubia*, *Pleuroxus hastirostris*), two copepods (*Acanthocyclops robustus* s.l., *Boeckella delicata*), one ostracod (*Eucypris virens*) and two rotifer species (*Filinia longiseta*, *Lepadella ovalis*) had been recorded in the treatment tanks, while only the cyclopoid copepod *A. robustus* s.l. was recorded in any of the control tanks. Species in the treatment tanks will primarily have emerged from diapausing stages in the sediments. For example, cladoceran species are often among the first taxa to hatch in diapause emergence experiments (Gyllström and Hansson 2004), but are typically not early components of pond colonisation studies, where early establishment is usually dominated by rotifer and cyclopoid copepod species (e.g., Jenkins and Buikema 1998; Cáceres and Soluk 2002; Frisch and Green 2007). The presence of *A. robustus* s.l. in the unseeded tanks at an early stage might be due to its transfer from treatment tanks or nearby ponds by, for example, birds, or other natural vectors (*A. robustus* s.l. was present in all treatment tanks at this time). The greatest accumulation rate of species in the seeded tanks occurred within the first 4 months of the experiment. This rapid acquisition of species suggests sediments containing diapausing zooplankton eggs are highly effective for accelerating community development.

At 12 months community composition and species richness differed greatly between most of the treatment and control tanks. Treatment tanks had generally acquired species adapted to pelagic conditions, such as the cladocerans *Ceriodaphnia dubia* and calanoid copepods *Boeckella delicata* and *Calamoecia lucasi*, whereas control tanks had a high proportion of small, benthic or littoral rotifer species (e.g., *Trichocerca pusilla*, *Lecane flexilis*, *Squatinella mutica* and bdelliods). The relatively low richness of rotifer species in the treatment tanks may be explained by the superior competitive abilities of the cladocerans in the treatment tanks. Both experimental (e.g., Gilbert 1988; MacIsaac and Gilbert 1991; Nandini et al. 2002) and field (Vanni 1986; Balvert et al. 2009) studies indicate that cladocerans can have profound effects on zooplankton community composition, and particularly on the presence of smaller species such as rotifers. Despite higher numbers of rotifer species in control tanks, at 12 months average species richness in the seeded tanks was significantly greater than that observed in the unseeded tanks. Species richness attained at 12 months in our study, with a standing mean of 2.6 species, was similar to the results of the pond colonization study by Cáceres and Soluk (2002), who found a standing mean richness of three species per pond after 1 year. However, these authors found a cumulative average of around ten zooplankton colonizers per tank after 12 months, higher than the average of five species in our tanks, while Jenkins and Buikema (1998) averaged approximately eight species per pond after 12 months. This variability is likely to have occurred due to, for example, distance to source populations (the experimental ponds of Cáceres and Soluk (2002) were in close proximity to other ponds), the diversity of zooplankton in local source ponds, pond design (Jenkins and Buikema's (1998) ponds were dug into the soil), and the availability or prevalence of vectors such as wind, rain or waterfowl (Jenkins and Underwood 1998).

Establishment rates of species intentionally introduced at 12 months were greater for the unseeded control tanks than they were for the seeded treatment tanks. Control tanks acquired five new species, while treatment tanks attained only two. Additionally, the number of treatment tanks where species established was far lower than for control tanks. Importantly, the non-indigenous North American calanoid copepod *Skistodiaptomus pallidus* established in seven of eight (88%) control tanks, but only in two of nine (22%) treatment tanks. Neither of these two treatment tanks, or any of the control tanks, had populations of the native calanoid copepods *Boeckella delicata* or

Calamoecia lucasi at 12 months. This supports the contention that calanoid copepods of similar size, or with significant dietary overlap, can not co-exist (e.g., Hutchinson 1967; Maly and Maly 1997). This also underlies the importance of biotic resistance as an important factor reducing the establishment of non-indigenous zooplankton, particularly for calanoid copepods, in New Zealand's constructed waters. New Zealand surveys of non-indigenous calanoid copepods, including *S. pallidus*, indicate these are all currently confined to constructed waters, such as farm dams, disused mine pits and ornamental ponds (Banks and Duggan 2009). This pattern, along with our finding that *S. pallidus* did not establish in any tanks where calanoid copepod populations were present at 12 months, suggests that reduced biotic resistance is a major factor responsible for the preferential establishment of non-indigenous zooplankton in New Zealand constructed waters. Overall, however, it is apparent that community composition, and not simply species richness, is likely important for reducing establishment rates.

Chydorus sp. was able to establish in a small proportion of the seeded treatment tanks. As the establishment of *S. pallidus* can be attributed to the absence of species with similar ecological requirements (i.e., other calanoid copepods), the establishment of *Chydorus* sp. in treatment tanks may also be due to similar reasons (i.e., a lack of other benthic/littoral chydorid cladocerans). This is also in agreement with Dzialowski (2010), who found that the presence of particular key species (e.g., the cladoceran *Daphnia magna*) provided resistance to the establishment of invaders (*Daphnia lumholtzi*, an invader of dams in USA) in experimental mesocosms. Overall, these results suggest that biotic resistance in zooplankton communities is strongest when there are species in the recipient community that have similar habitat requirements to those of the potential invaders. Because the dietary preferences of various zooplankton groups differ (DeMott 1986; Barnett et al. 2007), sediments containing diapausing stages of species that collectively exhibit a high diversity of dietary and habitat preferences will be most effective in accumulating biotic resistance against a variety of potential invaders. As such, for the effective acceleration of biotic resistance in new water bodies using sediment addition, the composition of the species within the sediments will be extremely important. However, care will be required regarding the source of the sediments to be used. Sediment would ideally be sourced from water bodies known to be free of non-indigenous species. However, alternative approaches include sourcing sediment from waters containing only non-indigenous species that are already ubiquitous in the area, which would undoubtedly arrive and establish in the new environment via other vectors, or screening sediments for eggs prior to addition. Also, as there is a strong trend for non-indigenous calanoid copepod species to establish in constructed waters in New Zealand, native calanoid copepods should be considered as a key taxon to be introduced to new or young water bodies there; however, as individual calanoid copepod species are known to have small, defined, native distributions in New Zealand (Banks and Duggan 2009), it would be inappropriate to include sediments from areas that will transplant species outside of their native ranges (thereby leading to intra-continental invasions). This latter example highlights the importance of local sourcing, or 'ecosourcing'. Such considerations are needed to ensure the integrity of local communities is maintained.

In our study, the species introduced in the lowest numbers (i.e., having the lowest propagule pressure) were generally the least successful at establishing populations. In general, higher propagule pressure increases the chances of successful establishment. *Trichocerca similis* and *Ascomorpha ovalis* were introduced to each tank as three and four individuals, respectively, and did not establish in any treatment or control tanks. *Chydorus* sp. had five individuals introduced into the tanks, and established in 38% of the control tanks and 22% of the treatment tanks, indicating that only a small number of individuals may be required for some species to establish (particularly if there are no ecologically equivalent species extant). The copepods *S. pallidus* and *M. leuckarti*, introduced in numbers of 16 and 25 respectively, had significantly higher success rates. These both established in 88% of the control tanks. However, in the treatment tanks, *S. pallidus* established in 22% of the tanks, with *M. leuckarti* failing to establish at all, indicating that an interaction exists between the propagule pressure and biotic resistance (e.g., von Holle and Simberloff 2005; Thomsen et al. 2006). Overall, it is likely that if propagule supply is high enough, even diverse communities may be invaded. As such, increased biotic resistance through seeding of constructed water bodies should not be considered a panacea, and efforts should still be made to reduce propagule supplies to these waters.

Conclusions

Our results indicate that introducing native propagules into new or young constructed water bodies will lead to an acceleration of zooplankton community development. Species established in significantly fewer seeded tanks, providing further evidence on the role of biotic resistance in reducing establishment rates of non-indigenous zooplankton. The environmental conditions among tanks seemingly allowed equal opportunities for species establishment over both experimental groups; indeed, the environmental factors that varied most greatly between the control and treatment groups, chlorophyll *a* and DO concentration, were more likely a function (rather than a cause) of the biota that resulted from sediment addition. The greater establishment rates between experimental groups could only be explained by the intentional seeding of the treatment tanks at the start of the study. At a global level, the extensive number and extent of constructed water bodies suggests that there are many water bodies that will exhibit low biotic resistance, and are therefore vulnerable to invasions. Although other explanations for higher establishment rates of non-indigenous zooplankton in constructed waters, such as disturbance (Havel et al. 2005; Johnson et al. 2008), may indeed hold true in some systems (i.e. dams), the discovery of non-indigenous calanoid copepod species in relatively undisturbed constructed water bodies in New Zealand infers that biotic resistance better explains the higher rates of invasion in many constructed habitats. We believe the addition of native species to constructed water bodies to increase establishment rates of native species, stimulate biotic resistance, and repel potential future invaders, should be implemented internationally. Efforts such as these should be applied to new water bodies before establishment of non-indigenous species has occurred. Due to the increasing rate of human mediated invasions, it is recommended that the addition of native species to constructed waters is implemented with a sense of urgency, as this will increase the effectiveness of this tool.

The implications of this study are widespread. Seeding of constructed waters should be feasible for all types of constructed waters (e.g., dams, ornamental ponds, retired mines). Due to low biotic resistance during the early stages of community development, establishment will likely require only small numbers of initial colonisers, particularly for species that reproduce by parthenogenesis (e.g., rotifers, cladocerans). For example, most species that colonise new lakes and ponds naturally will typically only arrive in low numbers with wind, rain and by waterfowl (e.g., Jenkins and Underwood 1998), and low propagule supplies are also likely involved in the introduction of most invaders. However, sexually reproducing species, such as calanoid copepods, may be disadvantaged in reservoirs that have low residence time, or that are large, as probabilities of meeting potential mates will be reduced. Such sexually reproducing species typically do not establish in ponds during short-term colonisation studies (Jenkins and Buikema 1998; Cáceres and Soluk 2002; this study). As such, we recommend common sense approaches, such as seeding floodplain ponds or backwaters where water movement is low in reservoirs, to facilitate species establishment. Limited opportunities to test these methods on new lakes, and the variety of potential environmental and biotic conditions encountered among lakes, will mean that an element of trial-and-error is required for undertaking this method. As establishment should occur even using small propagule supplies during the early stages of community development, the volumes of sediment required will not lead to increases in sedimentation, toxicants or nutrients to the system. However, the appropriate volumes require assessment. Small ponds with high retention times are likely to require significantly smaller volumes of sediment (a few hundred grams) than reservoirs that are larger or have lower retention times (perhaps several kilograms). The effectiveness of this technique for other taxa shown to have higher invasion rates in constructed waters (e.g., plants, macroinvertebrates and fish; Johnson et al. 2008) also deserves urgent attention. Nevertheless, our experiments indicate this method has the potential to be utilised in aquatic environments for the reduction of zooplankton invasions on an international scale.

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