Interaction between non-native predatory fishes and native galaxiids (Pisces: Galaxiidae) shapes food web structure in Tasmanian lakes

Nicolás Vidal^{1,2,7*}, Carolina Trochine^{3*}, Susanne L. Amsinck¹, Leon A. Barmuta⁴, Kirsten S. Christoffersen⁵ Marc Ventura⁶, Teresa Buchaca⁶, Frank Landkildehus¹, Scott A. Hardie⁴, Mariana Meerhoff^{7,1} and Erik Jeppesen^{1,2}

1) Department of Bioscience, Aarhus University, Vejlsovej 25, 8600 Silkeborg, Denmark

2) Sino-Danish Centre for Education and Research (SDC), Beijing, China

3) Departamento de Ecología, INIBIOMA CONICET-Universidad Nacional del Comahue, Quintral 1250, 8400 Bariloche, Argentina;

4) School of Biological Sciences, University of Tasmania, PB55, Hobart, Tasmania, Australia 7001

5) Department of Biology, Universitetsparken 4, 2100 Copenhagen Ø, Denmark

6) Centre for Advanced Studies of Blanes, Spanish National Research Council (CEAB-CSIC), 17300 Blanes, Catalonia, Spain

7) Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este, Universidad de la República. Tacuarembó s/n Maldonado 20000, Uruguay

*Corresponding authors: Nicolás Vidal, e-mail: ocin101@hotmail.com; Carolina Trochine, e-mail: trochine@comahue-conicet.gob.ar

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Abstract

Non-native fish invasions threaten native fauna and even ecosystem functioning, not least in isolated island lakes. In Tasmania, where the native fish fauna is mostly composed of galaxiids, nine non-native freshwater fish species have been introduced over the past 150 years, with unclear ecological outcomes. We evaluated the effects of non-native predatory fishes (NNPF), environmental and various biological variables on the trophic niche of the native fish (galaxiids) and the potential cascading effects on water clarity. We analysed food web metrics (Layman's) based on both stable isotope ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) and fish stomach content in 14 Tasmanian shallow lakes along a NNPF abundance gradient. The food web metrics: range of $\delta^{13}\text{C}$ (CR) and $\delta^{15}\text{N}$ (NR) centroid distance (CD), and standard ellipse area (SEA) were calculated. Our results showed a negative relationship of NNPF relative abundance (CPUE) with the galaxiids trophic niche (i.e., CR_G , NR_G and CD_G , trophic position (Tpos_G) and the pelagic contribution to the diet (Pel_cont_G). Moreover, the proportion of galaxiids in the diet of NNPF was higher in turbid lakes. The zooplankton SEA was negatively correlated with the Pel_cont of NNPF diet, and its CPUE was positively related with the maximum body size of calanoid copepods. While our results suggest a negative effect of NNPF on the trophic niche of galaxiids, the cascading effects on phytoplankton biomass were weak. Non-native predatory fish may affect native fish prey and the outcome of these interactions needs to be considered for conservation purposes, particularly for island lakes, such as in Tasmania.

Introduction

Fish are major determinants of the food web structure and dynamics in aquatic systems, particularly in shallow lakes (Jeppesen et al. 1997 a,b). Fish stocking is a long-standing, popular and often successful management tool used for commercial or recreational fishing (Wiley 2006, Gozlan 2008), but it may have strong ecosystem effects. The effects of the introductions vary considerably, from no effects on the abundance and relatively weak effects on the size distribution of the native fish populations (Nasmith et al. 2010) to substantial effects on both their abundance and size structure, occasionally leading to local extinction (Penczak 1999, Menezes et al. 2012). Native fish species have been shown to be negatively affected by predation as well as competition with introduced non-native species (McIntosh et al. 1992, Macchi et al. 1999).

The mechanisms involved are competition for space and food and changes in behaviour in order to reduce the encounter probability with the predator (Brönmark and Hansson 2000). Such changes may require prey to select less favorable habitats (McIntosh et al. 1992, Stuart-Smith et al. 2008) or a reduction of their foraging activity level (Milano et al. 2010), which, in turn, may affect the food web structure and trophic position of the native fish (Vander Zanden et al. 1999) or their trophic niches (Elgueta et al. 2013). The effects of the non-native species can thus be complex and appear at several ecological levels (Simon and Townsend 2003, Townsend 2003), ranging from the individual level, for instance by altering the foraging behaviour (Milano et al. 2010), to the ecosystem level, for example by influencing nutrient and energy fluxes (Vitousek 1996) and the overall ecological state of the system (e.g. clear versus turbid) (Tronstad et al. 2011).

Anti-predator behaviour is energetically costly, with prey facing trade-offs between the benefits of reduced predation risk and mortality, and the potentially reduced fitness associated with feeding or mating (Lima and Bednekoff 1999, Preisser et al. 2005). Energetically optimal

behaviours may vary with environmental variables such as water transparency, being important for visual predators (De Robertis et al. 2003) such as trout (Hunt and Jones 1972). Turbid waters may affect the distance at which prey are located by a predator and its risk of predation (Abrahams and Kattenfeld 1997, De Robertis et al. 2003, Pekcan-Hekim et al. 2013). Increased turbidity may diminish the capability of visual predators to detect prey (Snickars et al. 2004, Skov and Nilsson 2007), Environmental variables also play a role in shaping lake food webs; not least lake size and productivity may control food web width and length, thereby affecting the trophic position of top predators (Post et al. 2000, Takimoto et al. 2012).

In islands, with few and sometimes isolated endemic species, the effects of introduced non-native fishes can be more severe than in the majority of continental lakes, everything else being similar. In Tasmania, stocking of non-native fish species has been a common practice for more than 150 years and some studies have shown negative effects on native fish communities in lakes and streams (Lintermans 2004, Hardie et al. 2006). Nine non-native fish species have been introduced in total to the island's freshwaters, mostly since the beginning of the last century (Lintermans, 2004). These are mainly salmonids such as brown trout (*Salmo trutta*) derived from the northern hemisphere, mainly Europe (Hardie et al. 2006). The success of non-native fish invasions may be linked to a low niche similarity with the native fish species, based on the assumption that invaders with low overlap with native species along several niche axes may more easily establish (MacArthur and Levins 1967). Moreover, functionally distinctive invaders are often the most ecologically damaging (Ricciardi & Atkinson 2004).

In Tasmania, most of the native fish fauna (64%) belong to the family Galaxiidae (16 species). Their larvae and juveniles use pelagic environments and feed on zooplankton (Modenutti et al. 1993), whereas adults use littoral-benthic and epibenthic environments to feed and spawn (Humphries 1989, Barriga et al. 2002, Rowe et al. 2002, Stuart-Smith 2006). Moreover, galaxiids

have a circumpolar distribution restricted to the southern hemisphere and have evolved isolated from large predatory fishes, and they are therefore particularly vulnerable to invading predatory fish (McDowall 2006, Hardie et al. 2006, Correa et al. 2012). In Tasmania, some galaxiid species have restricted geographical distributions and 11 species are endemic to the island, increasing their vulnerability and the need for conservation measures (Hardie et al. 2006). Their conservation status is also affected by other factors such as hydrological manipulations (Hardie 2007).

Remarkably little is known about the potential effects of non-native predatory species on the trophic niche of galaxiids and the cascading effects on lower trophic levels. Cascading effects as a consequence of trout invasion have been described in several streams (Simon and Townsend 2003). While a study conducted in mainly nutrient-poor Faroe Island lakes showed a relatively weak cascading effect of brown trout (Amsinck et al. 2006), removal of this species in a mesotrophic reservoir in New Zealand led to a reduction of the size distribution of zooplankton. This indicates that brown trout may have a cascading effect mediated by changes in prey fish and suggests an enhanced predation pressure of galaxiids on zooplankton when trout are absent (Duggan et al. 2015). Concurrently, an increase in zooplankton body size and a reduction of phytoplankton were detected after the introduction of lake trout (*Salvelinus namaycush*) in Yellowstone Lake, pointing to a reduction of the predation pressure on zooplankton by the native cutthroat trout (*Oncorhynchus clarkii henshawi*) (Tronstad et al. 2011).

We analysed the potential effects of non-native predatory fishes on the food webs in a series of Tasmanian lakes and evaluated the potential of cascading effects on zooplankton and phytoplankton. We explored their interactions with native galaxiids and how environmental characteristics of the lakes modulate such interactions by use of trophic niche metrics. We expected that the potential effects of non-native predatory fish density would be more important than the potential effects of environmental variables on the use of the resources measured as trophic niche

metrics and also the pelagic contribution to the diet of native galaxiids. A similar pattern was expected considering the entire food web; thus, top-down cascading effects on plankton were hypothesised. We further anticipated that the impact of non-native predatory fish on native prey would be less strong in turbid lakes where predation effects might be reduced due to lower visibility.

Materials and methods

Study area

Tasmania is the main island of a group of islands located 240 km south of the Australian continent (42°04'S, 146°35' E) (Fig. 1). It has a surface area of 64,519 km² and is characterised by a cool temperate climate with four defined seasons. Mean annual minimum temperatures range from below -3° C, on the Central Plateau, to 6° C on the coastal margins, and mean annual maxima range from 9° C to >20° C (source: standard 30-year climatology, Bureau of Meteorology, Australia, www.bom.gov.au). Geologically, the island is dominated in the west by Precambrian quartzite and by Jurassic dolerite in the centre and east. The prevailing westerly weather systems interact with the higher relief western mountains and Central Plateau to produce a strong gradient in mean annual rainfall, ranging from >3000 mm in the west to <400 mm in the Midland region. Vegetation varies from grasslands and open woodlands in the dry Midlands, eucalypt forest, alpine heathlands and large areas of cool temperate rainforests and moorlands in the rest of the island (Reid et al. 1999).

Sampling

We sampled 14 lakes in the austral summer 2007, from the 13th to the 27th of January. The lakes were located along a longitudinal gradient from east to west (Fig. 1) and covered a range of

limnological characteristics (Table 1), although most of them were shallow. We took a depth-integrated water sample around midday at the deepest point of each lake to quantify nutrient concentrations and phytoplankton chlorophyll *a* (Chl-*a*). The samples were brought to the laboratory in thermally insulated containers. Additionally, we collected zooplankton in a depth-integrated sample (2-20 L depending on average lake depth) that was filtered through a 20- μ m mesh net, back washed into filtered water and fixed with 4% Lugol's solution. We also recorded Secchi depth and profiles of temperature, dissolved oxygen, pH and conductivity *in situ* using handheld instruments.

Fish were sampled with Lundgren multi-mesh gill-nets and fyke-nets; the latter to include species that are not easily captured in gill-nets (e.g. short finned eel, *Anguilla australis*, Richardson). Each gillnet consisted of 14 sections (3 m wide and 1.5 m deep) with the following mesh sizes: 6.25, 8, 10, 12.5, 16.5, 22, 25, 30, 33, 38, 43, 50, 60 and 75 mm (knot to knot). Two to four gill-nets, depending on lake size, were set overnight (for a total of 17 to 20 hours), half in the littoral and half in the pelagic zone, while a fyke-net was placed near to the shore for the same period of time as the gill-nets. The fyke nets had an 8 m long central leader and a height of 0.6 m, and two internal valves (0.55 m). The nets were made of 8 mm mesh (knot to knot) except at the end of the funnel where the mesh was 4 mm; no bait was placed in the nets. Relative abundance of fish was calculated as CPUE (Catch Per Unit of Effort, i.e. number of fish per gill-net⁻¹ hour⁻¹ or number of fish per fyke-net⁻¹ hour⁻¹). We measured (0.1 cm total length) and weighed (0.01 g fresh weight) all fishes. We analysed stomach content and stable isotopes composition in a subsample of 15 individuals of each species, representative of the size range. Fish stomachs were removed and preserved in 96% ethanol, and muscle samples were taken and frozen (-18 °C) immediately after capture for posterior laboratory analysis.

For stable isotope analysis, we further collected mid-lake samples of: (1) zooplankton and pelagic macroinvertebrates, using vertical nets with two mesh sizes, 140 μm and 500 μm , so as to include organisms of different sizes, (2) littoral macroinvertebrates, using a kick-net (1000 μm mesh size) in the littoral zone, and (3) benthic macroinvertebrates from the deep zone, using a KC epibenthic sledge. In lakes where macrophytes were abundant, macroinvertebrates from the macrophyte beds were also collected with a sweep net. We collected three replicates of each item, and the samples were frozen immediately ($-18\text{ }^{\circ}\text{C}$) until analysis in the laboratory.

The fish and other communities were sampled following the National Parks and Reserved Land Regulations (1999) and Crown Lands Regulations (2001). The permit to collect wildlife for scientific purposes (permit N°FA06595) was issued by the Department of Primary Industries and Water.

Sample analyses

The Chl-*a* concentration was used as a surrogate for phytoplankton biomass. Depending on cell densities, a water sample between 100 ml and 1000 ml was filtered through Whatman GF/C filters (47 mm in diameter). Chl-*a* was determined spectrophotometrically after ethanol extraction (Jespersen and Christoffersen 1987). Total phosphorus (TP) was determined as molybdate reactive phosphorus (Murphy and Riley 1962) following persulphate digestion (Koroleff 1970) and total nitrogen (TN) as nitrite after potassium persulphate digestion (Solarzano and Sharp 1980).

Zooplankton individuals were identified to species level and counted using a stereomicroscope. We measured a subsample of 20-30 randomly selected individuals per species (to 0.01 mm) using an ocular scale and calculated biomass using published length-weight relationships (McCauley 1984).

The stomach content of fishes was analysed under a stereomicroscope, all prey being counted and identified to the lowest possible taxonomic level. The absolute volume of each food item was measured using standardised Hyslop's indirect volumetric method (Hyslop, 1980) to

calculate its proportion in the stomach content. The prey were grouped into nine categories: zooplankton (including copepods, *Daphnia sp.* and other cladocerans), benthic crustaceans (amphipods and isopods), benthic insects (Trichoptera, Oniscigastridae, Leptophlebiidae, Gripopterygidae, Dytiscidae, Chironomidae, Zygoptera, Anisoptera and Ceratopogonidae), planktonic insects (pupae and nymphs of the aquatic insects Notonectidae and Corixidae), benthic molluscs (*Pisidium sp.*, *Glasidorbis sp.* and *Isidorella sp.*), terrestrial insects (Coleopteran, Hemipteran, Hymenoptera, Acrididae, Blatodea and Lepidoptera), fishes to species level, unidentifiable animal remains and unidentifiable macrophytes.

The samples for stable isotope analyses were defrosted in the laboratory and cleaned using distilled water. We separated and identified macroinvertebrates to family level and cladocerans to genera level, while copepods were separated into cyclopoids and calanoids. The processed samples were freeze-dried, fine-powdered and kept in glass vials and then weighed using a scale precision of 0.01 mg (Sartorius Genius), loaded into tin capsules and analysed at UC Davis Stable isotope Facility (University of California, USA) for stable isotopes of carbon (^{12}C : ^{13}C , $\delta^{13}\text{C}$) and nitrogen (^{14}N : ^{15}N , $\delta^{15}\text{N}$). We expressed stable isotope data in parts per thousand (‰) deviations from international standards (Vienna Pee Dee Belemnite and atmospheric N_2 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) using the following equation:

$$\delta X = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$$

where $X = ^{13}\text{C}$ or ^{15}N and R = ratio of heavy/light isotope content ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$). Internal precision was <0.2‰.

Food web metrics and mixing models

Food web metrics as defined by Layman et al. (2007) were calculated using the package SIAR (R Core Team, 2014). The metrics used were: the range of $\delta^{13}\text{C}$ (CR), representing the width

of the food web and the range of $\delta^{15}\text{N}$ (NR), indicating the height of the food web. Both ranges are calculated as the difference between the maximum and minimum values in the food web. Mean centroid distance (CD) is a measure of the extent of dispersal of the species or groups in the bi-plot and thus represents trophic diversity. Since the total area of the food web (TA) metric is affected by extreme values and therefore increases with sample size (Jackson et al., 2011), we calculated the standard ellipses areas with and without correction for sample size, (SEAc) and (SEA), respectively, using the package SIBER (R Core Team, 2014) to compare the food webs between lakes. SEAc and SEA offer a better and more comparable description of the isotopic niche than the TA. Standard ellipses represent bivariate data as SD is used in univariate cases to represent the isotopic niche width instead of convex hulls (Batschelet, 1981).

Layman's metrics calculated using stable isotope signatures of primary producers may be highly variable, therefore the use of stable isotope values of primary consumers is preferred because they may better integrate the natural variation in space and time of basal resources (producers) (Layman et al. 2007). Layman's metrics have been criticised for lacking scale, which may affect their interpretation if basal resources among the different water bodies compared are not homogeneous (Hoeinghaus and Zeug 2008). The difference between the carbon values of basal resources in the pelagic and the littoral zone was not constant among the lakes in our dataset (Appendix 1). To avoid misleading results, we standardised the CR of galaxiids (CR_G) and of the entire food web (CR_E) by dividing these values by the CR of all potential resources for galaxiids (CR_S) (calculated as CR pelagic resources – CR of littoral resources) in each lake. Then, the $\text{CR}_G:\text{CR}_S$ and $\text{CR}_E:\text{CR}_S$ ratios represented the trophic niche of galaxiids and the entire food web, respectively, independently of the heterogeneity of the basal resources among lakes.

We also estimated the trophic position of each individual using the following equation (Post 2002):

$$\text{Trophic position}_{\text{consumers}} = ((\delta^{15}\text{N}_{\text{consumers}} - \delta^{15}\text{N}_{\text{base}})/2.98) + 2$$

where $\delta^{15}\text{N}_{\text{consumers}}$ are the isotopic values of each individual analysed and $\delta^{15}\text{N}_{\text{base}}$ corresponds to baseline organisms (i.e. herbivorous invertebrates with the lowest $\delta^{15}\text{N}$ value), 2.98 is the expected $\delta^{15}\text{N}$ fractionation per trophic level (Vanderklift and Ponsard 2003) and 2 is the theoretical trophic level of baseline organisms. The trophic position (Tpos, based on $\delta^{15}\text{N}$ values) and $\delta^{13}\text{C}$ values for all available organisms were plotted in order to show the food web structure of each lake following Fry (1991).

To elucidate the relative importance of littoral and pelagic contributions to the fish diet, particularly that of galaxiids, we used a Bayesian-based mixing model of two sources (Parnell et al. 2010) included in the R package SIAR (Parnell et al. 2008). For this, we used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of fish muscle as the consumers signal and littoral herbivorous macroinvertebrates as the littoral signal, while zooplankton (cladocerans, cyclopoid and calanoid copepods) were used as the pelagic signal.

Data analysis

We first performed an ordination analysis, PCA, on limnological variables (package Stats, R Core 2017). We examined the potential effects of the relative abundance of the non-native predatory fish (CPUE), plus several environmental variables (i.e. altitude, lake area, conductivity, pH, oxygen, Secchi depth, temperature, Chl-*a*, TN and TP) and biological variables (zooplankton to phytoplankton ratio, pelagic zooplankton, calanoid maximum size, cladoceran maximum size) on the food web metrics of native galaxiid (Layman's metrics, calculated including only galaxiids: standardised carbon range (CR_G: CR_S), nitrogen range (NR_G), centroid distance (CD_G) and trophic position (Tpos_G) as well as the pelagic contribution to their diet (Pel_cont_G)) and the effects on the entire food web metrics (Layman's metrics, calculated including the following components of the

food web: macroinvertebrates, zooplankton and the fish community). For these purposes, we applied the best subset function to obtain the best models (leaps package for R by Lumley 2017). We then selected the final model based on different estimators, such as adjusted R^2 , Bayesian Information Criterion and Mallow's cp and also considering the variance inflation factor ($VIF < 5$) of the selected variables (car package for R by Fox and Weisberg 2011). We used ANOVA to test whether the reduction in the residual sum of squares between the full model and the selected model was statistically significant.

Moreover, the direct predatory effect of non-native predatory fish was measured as the proportion of galaxiids in the diet of non-native predatory fish. We additionally analysed the strength of the trophic cascading effects produced by the non-native predatory fish on the body size of zooplankton and the SEA and SEAc of the food web of the zooplankton community, the zooplankton biomass:phytoplankton biomass ratio ($\text{Chl-}a \text{ } \mu\text{g L}^{-1}$ was multiplied by 66 to convert it to algal dry mass $\mu\text{g L}^{-1}$ (Jeppesen et al. 1998)) and $\text{Chl-}a$:nutrient (TN and TP) ratios were tested using linear regressions after checking for normality of the data (the ratios of zooplankton:phytoplankton biomass and $\text{Chl-}a$:TN concentrations were log transformed).

Results

Lake characteristics, zooplankton community and fish capture

The sampled lakes comprised a wide range of trophic status, ranging from oligotrophic, with $0.002 \text{ mg TP L}^{-1}$, to eutrophic, with $0.122 \text{ mg TP L}^{-1}$. The lakes also varied in surface area (1.3-4433.0 ha) and maximum depth (0.5-15.0 m) across altitudes ranging from 530 to 1160 m.a.s.l. (Table 1). The ordination analysis showed that variables related to productivity such as $\text{Chl-}a$, TN, TP and conductivity were negatively associated with the first axes (explaining 44.8 % of variance).

Lagoon of islands, Lakes Sorell and Tooms showed high values of Chl-*a*, TN and TP.

Morphometric variables such as depth, area and altitude were related to the second axes (explaining 20.2 % of variance) and characterised Lakes Howes Bay, Augusta, Ada and Carter. Lakes Selina, Echo and Dora were characterized by high depth (see Appendix 2).

Zooplankton biomass varied among the lakes from 5 to 198 μg dry weight L^{-1} (Table 2). In most of the lakes, the zooplankton community was dominated by calanoid copepods (Table 2), representing more than 50% of the total biomass in 10 of the 14 lakes. The dominant species were *Boeckella rubra* Smith and *Calamoesia tasmanica* (Smith), while *Calamoesia ampulla* (Searle) and *Boeckella triarticulata* (Thompson) were less frequent (Table 2). The Cladocera were dominated by *Bosmina meridionalis*, which was present in all the sampled lakes, followed by *Ceriodaphnia sp.*; *Daphnia carinata* was present in low densities in only three lakes (Table 2).

A total of 11 fish species (see Appendix 3) were recorded: five galaxiids (*Galaxias auratus* Johnston, *G. truttaceus* (Valenciennes), *G. brevipinnis* Günther, *G. maculatus* (Jenyns) and *Paragalaxias julianus*; McDowall & Fulton), three trout species: *Salmo trutta*, *Oncorhynchus mykiss* (Walbaum) and *Salvelinus fontinalis* Mitchill; perch, *Perca fluviatilis* L.; eel, *Anguilla australis* and tench (*Tinca tinca* L.). All the non-native fish captured in this study were piscivorous: *S. trutta* (Stuart-Smith et al., 2004), *S. fontinalis* (Power et al., 2002) and *P. fluviatilis* (Collette et al., 1977), except *Tinca tinca* (Alaş et al., 2010).

Galaxiids were captured in nine lakes and varied in relative abundances, while non-native predatory fishes were captured in seven lakes that were naturally inhabited by galaxiids (Fig. 2). The highest total CPUE was recorded in Lake Sorell (1.50 fish $\text{net}^{-1} \text{hour}^{-1}$) followed by Lake Echo (1.42 fish $\text{net}^{-1} \text{hour}^{-1}$) and Tooms Lake (1.31 fish $\text{net}^{-1} \text{hour}^{-1}$). *Salmo trutta* were recorded in 10 lakes, with CPUE ranging from 0.16 fish $\text{net}^{-1} \text{hour}^{-1}$ in Lake Leake to 0.90 fish $\text{net}^{-1} \text{hour}^{-1}$ in Lake Ada. Other trout species with lower CPUE were *O. mykiss* in Lake Augusta, Penstock Lagoon and

Tooms Lake, and *S. fontinalis* in Lake Selina and Lake Leake. Galaxiids CPUE ranged from 0.01 fish net⁻¹ hour⁻¹ in Lake Echo to 0.97 fish net⁻¹ hour⁻¹ in Tooms Lake, and galaxiids were the only fish species found in Lake Dora (0.17 fish net⁻¹ hour⁻¹) and Little Dora (0.89 fish net⁻¹ hour⁻¹), the two lakes with the highest altitude. *Tinca tinca* appeared only in Bronte Lagoon, with 0.14 fish net⁻¹ hour⁻¹. Finally, *A. australis* was only captured in the fyke-nets, its CPUE ranging from 0.06 fish fyke-net⁻¹ hour⁻¹ in Lake Sorell and Bronte Lagoon to 1.16 fish fyke-net⁻¹ hour⁻¹ in Lake Leake.

The occurrence of the different fish species, particularly the absence of non-native predatory fish in the predator-free lakes, was also confirmed by literature review and unpublished data from local researchers (Scott Hardie and Leon Barmuta personal communication; see Appendix 3 for more details).

Stable isotopes

The mean raw isotopic values for the baselines ranged from -32.4 to -17.8 for $\delta^{13}\text{C}$ and from 1.0 to 5.1 for $\delta^{15}\text{N}$ in the pelagic zone, while in the littoral zone the values ranged from -28.5 to -14.7 for $\delta^{13}\text{C}$ and from -1.3 to 6.7 for $\delta^{15}\text{N}$ (see Appendix 1).

Galaxiid food webs metrics

The CPUE of non-native predatory fish was included as a significant factor in all the selected models of galaxiid food web metrics (Table 3). Particularly, the regressions including just CPUE of non-native predatory fish were significant except for Pel_cont_G; the CPUE of the non-native fish were negatively related to all the Layman's metrics. The best model for Tpos_G indicated that this metric was negatively related to the CPUE of non-native predatory fish, Secchi depth and Chl *a* (Table 3). The NR_G values decreased as a linear function of CPUE (Fig. 3, Table 2). The models for the ratio CR_G:CR_S for galaxiids showed a negative relationship with CPUE and positive

relationship with oxygen concentration. The CD_G was negatively related to the CPUE of non-native predatory fish and positively to oxygen concentration and the maximum body size of calanoid copepods (Table 3). Finally, the best linear model for Pel_cont_G showed a negative relationship with the CPUE of non-native predatory fish, water depth and the zooplankton to phytoplankton biomass ratio (Table 3).

Accordingly, the configuration of the food webs, in particular the food web of galaxiids, differed between the lakes containing non-native predatory fish and the lakes without them (Fig. 4). A direct effect of predation, measured as the proportion of galaxiids in the stomach content of the non-native predatory fish, was previously observed using both visual inspection and DNA barcoding analyses in a subset of the studied lakes (Jo et al., 2016). A stronger direct effect of predation was observed in the turbid lakes, Tooms (42.7%) and Sorell (21.9%), and in the humic Lake Selina (25%) than in the clear lakes containing galaxiids (0-10.2%). Moreover, a significant positive relationship between the proportion of fish in the diet of non-native predatory fish and phytoplankton Chl-*a* was observed ($R^2=0.64$; $p<0.05$). In contrast, we found a negative relationship between Secchi depth and the proportion of pelagic prey (including planktonic zooplankton: *Daphnia* sp., *Bosmina* sp., *Diaphanosoma* sp., copepods and planktonic invertebrates: such as corixidae and insect pupae) in the stomach content of the analysed galaxiids (Fig. 5).

Entire food web metrics

For the entire food web, CPUE of non-native predatory fish did not appear as a significant factor in any of the models explaining the variability of the food web metrics (Table 4). The $CR_E:CR_S$ ratio increased as a linear function of cladoceran maximum body size ($clad_max_size$) (Table 4). SEA_E of the entire food web was positively related to the water temperature and CPUE of all fish and negatively to calanoid maximum body size, while $SEAc_E$ was only significantly and

positively related to water temperature (Table 4). Pel_cont_E decreased with Secchi depth (Table 4), while the metrics related to the height of the entire food web (i.e. Tpos and NR) were not correlated with any of the environmental or biological variables.

Potential predation pressure indicators and cascading effects

The mean body size of cladocerans was not related to the CPUE of non-native predatory fish or to the pelagic contribution to the fish diet (Fig. 6a and 6b). While the maximum size of calanoid copepods showed a positive relationship with the CPUE of non-native predatory fish (Fig. 6c), the SEAz of the zooplankton community was negatively related to the pelagic contribution to the diet of fish, encompassing all species (Fig. 6d). The top down proxies, i.e. the zooplankton:phytoplankton biomass ratio as well as the Chl-*a*:TN and Chl-*a*: TP ratios, were not related to the CPUE of non-native predatory fish or to the pelagic contribution to the diet of fish (see Appendix 4).

Discussion

We studied the interaction between the non-native predatory fish and the native fish fauna (galaxiids) in 14 Tasmanian lakes. Our results indicate strong effects on galaxiids, and both direct and indirect effects were observed. Direct lethal effects were revealed by the presence of galaxiids in the guts of non-native predatory fish, whereas nonlethal effects (e.g. reduction of trophic niche) were evidenced as most of the food web metrics calculated for galaxiids (e.g. NR_G, Tpos_G, CR_G: CR_S, and CD_G) decreased with the increase in the relative abundance of non-native predatory fish, suggesting a reduction of the trophic niche of the native fish fauna.

The food web metrics of galaxiids showed higher values in the lakes without non-native predatory fish (i.e. Lake Dora and Lake Little Dora) than in lakes with non-native predatory fish (Fig. 4) regardless of differences in other lake characteristics. Comparable reductions of trophic

height have been observed for galaxiids in trout-invaded Patagonian lakes in South America (Correa et al., 2012). Correa et al. (2012) cited two alternative and non-exclusive hypotheses for such trophic niche shifts of galaxiids: the exploitative competition hypothesis, based on changes in available food, and the predator avoidance hypothesis, based on predator-induced modifications of feeding behaviour. Although we cannot readily disentangle these two mechanisms in our data, the predator avoidance hypothesis has received much more support for galaxiids (Fraser and Huntingford, 1986, Stuart-Smith et al. 2008, Milano et al. 2010). Galaxiids, as many other small fish species, typically use physically complex habitats (e.g. macrophyte beds or rocks) as refuges against predators (Stuart-Smith et al. 2008). Behavioural experiments have demonstrated that in the presence of non-native predatory fish, galaxiids alter their patterns of habitat use and tend to spend less time in open waters. Instead, galaxiids increase their presence within macrophyte beds or among rocks, while simultaneously reducing their swimming activity (Stuart-Smith et al. 2008, Milano et al. 2010) or altering their feeding patterns in the presence of non-native predatory fish (Fraser and Huntingford, 1986, Fraser and Gilliam, 1987). By restricting their feeding in time or use of space, native fish may reduce the width of their trophic niche, as was observed in our study. These changes in the use of resources may also explain why the galaxiid metrics linked to trophic niche such as ($CR_G:CR_S$) and trophic diversity (CD_G), were negatively related to the abundance of the non-native predatory fish. Using an experimental approach, Stuart-Smith et al. (2007) found no effect on the foraging success of *Galaxias auratus* of increasing use of complex habitats in the presence of predators. However, our results indicate a reduced niche width of native galaxiids (NR_G and $Tpos_G$) in the presence of non-native predatory fish.

Besides, our results suggest that water transparency negatively affected $Tpos_G$, with galaxiids reaching higher $Tpos$ in turbid environments. Furthermore, Pel_cont_G was negatively related to the CPUE of non-native predatory fish and the zoo:phyto biomass ratio, suggesting that

the pelagic resources are less used by galaxiids in the presence of predators, but that their use increases with the increase in pelagic biomass. A potentially higher pelagic productivity (using phytoplankton biomass as proxy) could directly increase the availability of pelagic resources. Alternatively, an increase in water turbidity may reduce the capture efficiency of the non-native predatory fish. As a result, the foraging strategy of galaxiids may shift: in turbid environments galaxiids could use the open water areas more. Apparently, an increase in lake trophic state due to enhanced external nutrient loading could indirectly favour the galaxiids and, consequently, reduce the indirect effects of predation. However and contrary to our expectations, the direct effect of the non-native predatory fish (measured as the proportion of galaxiids in the diet of non-native predatory fish) appeared to be stronger in the turbid and the humic lakes than in the clear water lakes, indicating that predation on galaxiids occurred despite the reduced visibility. Stuart-Smith et al. (2004) reported enhanced predation on *G. auratus* by brown trout in Lake Sorell, Tasmania, after six years of water transparency reduction. Additionally, galaxiids have evolved isolated from large predatory fishes and may potentially have produced a naïve and inappropriate anti-predator response, resulting in greater mortality due to non-native predatory fish (Cox & Lima 2006).

The structure of the entire food web appeared to be mainly influenced by environmental variables rather than the CPUE of non-native predatory fish. The calculated pelagic contribution to the fish diet increased with increasing water turbidity, as indicated by a negative correlation with Secchi depth. This is to be expected as turbid waters and high-nutrient lakes typically have a lower benthic production than clear lakes (Vadeboncoeur et al. 2001, Liboriussen and Jeppesen 2003). Consequently, fish are expected to rely more on pelagic food resources in such lakes. Furthermore, the $CR_E:CR_S$ ratio was positively related to the body size of cladocerans, which may be used as a proxy of pelagic food availability. The SEA and SEAc of the entire food web increased with increasing water temperature. Our results, therefore, indicate that environmental variables and the

availability of pelagic food affected the entire food web metrics more significantly than did the abundance of non-native predatory fish, pointing to a stronger bottom up control at the base of the food web in these systems.

Townsend (2003) observed trophic cascading effects of non-native predatory fishes in New Zealand streams; particularly that trout reduced the macroinvertebrate grazing pressure, thereby enhancing algal biomass and affecting algal species composition. In contrast, we found relatively modest cascading effects in the studied Tasmanian lakes. We observed an increase in the maximum body size of calanoid copepods with increasing abundance of non-native predatory fish; however, we did not find significant relationships with the mean body size of cladocerans. The different response among zooplankton groups may owe to the fact that calanoid copepods were dominant in most of the lakes and therefore likely more responsive to changes in the galaxiid predation pressure. When we analysed the effect on the zooplankton community from a functional perspective, the SEA generally declined with increased pelagic contribution to the diet of the fish (Fig. 6D), suggesting that the trophic diversity of the zooplankton community decreased due to fish feeding in the pelagic zone. The observed effect on the zooplankton community at different levels was not propagated to the lower trophic level since neither the zooplankton:phytoplankton biomass ratio or the Chl- α :nutrient ratio were related to the abundance of non-native predatory fish, suggesting absence of trophic cascading effects from top predators to primary producers in these Tasmanian lakes.

In conclusion, the introduction of non-native predatory fish could be considered an important threat with severe consequences for the native fish species of Tasmanian lakes and the overall functioning of the invaded lakes. Galaxiids, of which many species are endemic to Tasmania, are considered a threatened group (McDowall 2006, Hardie et al. 2006), and the present and earlier studies reveal direct and indirect effects of non-native predatory fish on galaxiids. Particularly, based on stable isotope and stomach content analyses, we document here a negative

relationship between non-native predatory fish abundance and the access to food resources by native galaxiids, leading to a reduction in trophic niche width and the trophic position of galaxiids, although the observed trophic cascading effects were rather weak.

In a recent work, Dawson et al. (2017) investigated the global hotspots of alien species richness worldwide, with special focus on the need to prioritise the prevention of further introductions of alien species to islands. Our results also emphasise the occurrence of indirect effects of non-native predatory fish introduction on the native fish fauna and ecosystem functioning in isolated landscapes such as Tasmanian lakes.

Supplementary material

Additional Supporting Information can be found in the online version of this article:

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FIGURES

Figure 1. Study area and location of sampled lakes across Tasmania, Australia.

Figure 2. Fish abundance in lakes containing galaxiids (n=9) given as Catch Per Unit of Effort (CPUE, fish net⁻¹ hour⁻¹) of non-native predatory fish (black) and native galaxiids (grey). Lakes are ordered by increasing CPUE of non-native predatory fish.

Figure 3. Linear regression between the CPUE of non-native predatory fish and Layman's metrics of galaxiids. Nitrogen range ($y = -2.8x + 2.7$; $R^2 = 0.87$; $p < 0.001$); carbon range ($y = -0.6x + 0.5$; $R^2 = 0.60$; $p < 0.05$) and centroid distance ($y = -1.0x + 1.2$; $R^2 = 0.53$; $p < 0.05$).

Figure 4. Food webs in lakes with contrasting non-native predatory fish: without predators, lakes Dora and Little Dora (A and B), and with non-native predatory fish, lakes Selina and Bronte Lagoon (C and D), including the basal resources (zooplankton and macroinvertebrates).

Figure 5. Proportion of pelagic prey in the stomach content of galaxiids (including planktonic zooplankton: *Daphnia* sp., *Bosmina* sp., *Diaphanosoma* sp., copepods and planktonic macroinvertebrates: corixidae and insect pupae) versus logarithm of lake Secchi depth (m).

Figure 6. Indirect effects of fish on zooplankton: Maximum body size of cladocerans versus CPUE of non-native predatory fish (A) ($y = 792.7x + 495.3$; $R^2 = 0.13$; $P = 0.22$; $n = 14$) and the pelagic contribution to the fish diet (B) ($y = -336.1x + 952.4$; $R^2 = 0.011$; $P = 0.81$; $n = 13$). Maximum body of calanoid copepods versus CPUE of non-native predatory fish in all lakes where calanoids were present ($y = 169.3x + 715$; $R^2 = 0.46$; $p = 0.002$; $n = 13$) (C), standard ellipses of the zooplankton trophic

webs (SEAz) versus the pelagic contribution to the fish diet, including all fish (D) ($y = -8.9x + 9.2$; $R^2 = 0.34$; $p=0.06$; $n=10$). Due to absence of basal resources, food web metrics could not be calculated for Penstock Lagoon, and it was therefore excluded from the analysis.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Supporting information

Appendix1.

Mean and SD of the stable isotopic composition of baseline resources from the pelagic and the littoral zones. Penstock Lagoon was excluded, due to absence of data for basal resources.

	Pelagic				Littoral			
	$\delta^{13}\text{C}$	SD	$\delta^{15}\text{N}$	SD	$\delta^{13}\text{C}$	SD	$\delta^{15}\text{N}$	SD
Lake Selina	-31.7 $\pm$ 1.1		3.6 $\pm$ 0.8		-26.2 $\pm$ 0.6		2.6 $\pm$ 1.8	
Lake Dora	-32.4 $\pm$ 1.0		3.2 $\pm$ 1.2		-28.5 $\pm$ 2.0		1.9 $\pm$ 1.5	
Lake Little Dora	-30.5 $\pm$ 0.7		2.1 $\pm$ 1.1		-25.9 $\pm$ 1.5		-1.3 $\pm$ 0.3	
Lake Ada	-23.9 $\pm$ 0.5		1.0 $\pm$ 0.7		-20.0 $\pm$ 1.6		1.8 $\pm$ 0.8	
Lake Augusta	-23.2 $\pm$ 1.1		4.2 $\pm$ 1.2		-17.2 $\pm$ 2.4		2.9 $\pm$ 1.3	
Carter Lakes	-17.8 $\pm$ 0.6		1.5 $\pm$ 0.5		-14.7 $\pm$ 1.1		2.3 $\pm$ 1.3	
Howes Lagoon Bay	-21.5 $\pm$ 0.0		3.9 $\pm$ 0.0		-16.9 $\pm$ 1.2		1.7 $\pm$ 0.5	
Bronte Lagoon	-31.0 $\pm$ 0.4		4.6 $\pm$ 0.4		-24.0 $\pm$ 3.8		3.7 $\pm$ 1.4	
Lake Echo	-29.5 $\pm$ 0.7		2.5 $\pm$ 0.4		-24.1 $\pm$ 0.4		6.7 $\pm$ 2.2	
Lagoon of Islands	-24.1 $\pm$ 0.0		4.0 $\pm$ 0.0		-20.7 $\pm$ 4.1		5.0 $\pm$ 1.2	
Lake Sorell	-26.6 $\pm$ 0.2		5.1 $\pm$ 0.3		-22.1 $\pm$ 1.1		6.4 $\pm$ 1.6	
Lake Leake	-25.6 $\pm$ 0.5		3.7 $\pm$ 0.3		-17.5 $\pm$ 1.8		3.0 $\pm$ 1.2	
Tooms Lake	-19.3 $\pm$ 1.6		4.4 $\pm$ 2.1		-18.8 $\pm$ 0.0		4.4 $\pm$ 0.0	

Appendix2.

Ordination analysis PCA of the environmental data measured in each lake: Alt, altitude; Chl *a*, Chlorophyll *a*; Temp, temperature; Cond, conductivity; TP, total phosphorus; TN, total nitrogen

Appendix3.

Abundance shown as Capture per Unit of Effort (CPUE net⁻¹ hour⁻¹) for the fish caught in the different lakes, ordered from west to east.

Lake name	Non-native					Native						Missing species based on literature and personal observation
	St	Om	Sf	Pf	Tt	A	Ga	Gt	Gb	Pj	Gm	
Lake Selina	0	0	0.1	0	0	0	0	0	0.37	0	0	No missing
Lake Dora	0	0	0	0	0	0	0	0	0.17	0	0	No missing
Lake "Little Dora"	0	0	0	0	0	0	0	0	0.89	0	0	No missing
Lake Ada	0.9	0	0	0	0	0	0	0	0	0	0	Pj, Gt, Gb
Lake Augusta	0.82	0.03	0	0	0	0	0	0	0	0.12*	0	Gt, Gb
Carter Lakes	0.5	0	0	0	0	0	0	0	0	0	0	Pj, Gt, Gb
Howes Lagoon Bay	0.5	0	0	0	0	0	0	0	0	0	0	Om, Pj, Gt, Gb
Bronte Lagoon	0.56	0	0	0	0.19	0.06*	0	0.22	0	0	0	Om, Gb
Lake Echo	0.18	0	0	1.22	0	0	0.01	0	0	0	0	Om
Penstock Lagoon	0.33	0.1	0	0	0	0	0	0.15	0	0	0	Pe, Pd, Gb, Aa
Lagoon of Islands	0	0	0	1.24	0	0	0	0	0	0	0	No missing
Lake Sorell	0.63	0	0	0	0	0.06*	0.87	0	0	0	0	Om, Cc
Lake Leake	0.16	0	0.03	0.47	0	1.16*	0	0	0	0	0	Om
Tooms Lake	0.31	0.19	0	0	0	0.56*	0	0	0	0	0.97	No missing

St (*Salmo trutta*), Om (*Oncorhynchus mykiss*), Sf (*Salvelinus fontinalis*), Pf (*Perca fluviatilis*), Tt (*Tinca tinca*), A (*Anguilla australis*), Ga (*Galaxias auratus*), Gt (*Galaxias truttaceus*), Gb (*Galaxias brevipinnis*), Pj (*Paragalaxias julianus*), Gm (*Galaxias maculatus*). **Pe** (*Paragalaxias eleotroides*), **Pd** (*Paragalaxias dissimilis*) and **Cc** (*Cyprinus carpio*). CPUE corresponds to the average of the number of individuals captured per net per hour, *corresponds to capture per fyke net per hour.

Lake name	Non-native					Native					
	St	Om	Sf	Pf	Tt	A	Ga	Gt	Gb	Pj	Gm
Lake Selina	0	0	0.10	0	0	0	0	0	0.37	0	0
Lake Dora	0	0	0	0	0	0	0	0	0.17	0	0
Lake "Little Dora"	0	0	0	0	0	0	0	0	0.89	0	0
Lake Ada	0.90	0	0	0	0	0	0	0	0	0	0
Lake Augusta	0.82	0.03	0	0	0	0	0	0	0	0.12*	0
Carter Lakes	0.50	0	0	0	0	0	0	0	0	0	0
Howes Lagoon Bay	0.50	0	0	0	0	0	0	0	0	0	0
Bronte Lagoon	0.56	0	0	0	0.19	0.06*	0	0.22	0	0	0
Lake Echo	0.18	0	0	1.22	0	0	0.01	0	0	0	0
Penstock Lagoon	0.33	0.1	0	0	0	0	0	0.15	0	0	0
Lagoon of Islands	0	0	0	1.24	0	0	0	0	0	0	0
Lake Sorell	0.63	0	0	0	0	0.06*	0.87	0	0	0	0
Lake Leake	0.16	0	0.03	0.47	0	1.16*	0	0	0	0	0
Tooms Lake	0.31	0.19	0	0	0	0.56*	0	0	0	0	0.97

St (*Salmo trutta*), Om (*Oncorhynchus mykiss*), Sf (*Salvelinus fontinalis*), Pf (*Perca fluviatilis*), Tt (*Tinca tinca*), A (*Anguilla australis*), Ga (*Galaxias auratus*), Gt (*Galaxias truttaceus*), Gb (*Galaxias brevipinnis*), Pj (*Paragalaxias julianus*), Gm (*Galaxias maculatus*). CPUE corresponds to the average of the number of individuals captured per net per hour, *corresponds to capture per fyke net per hour.

Appendix 4.

Indicators of cascading fish effect on zooplankton and phytoplankton related to the abundance (CPUE) and diet (pelagic contribution to diet) of non-native predators in all lakes. The indicators are: the zooplankton:phytoplankton biomass ratio (Zoo:Phyto ratio), chlorophyll-*a*:total phosphorous ratio (Chl-*a*:TP ratio) and chlorophyll-*a*:total nitrogen ratio (Chl-*a*:TN ratio). Penstock Lagoon was excluded from the analysis as the pelagic contribution to the fish diet could not be calculated due to absence of data for basal resources.