

# Rainbow trout effects on zooplankton in the reproductive area of the critically endangered hooded grebe

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## ABSTRACT

1. Aquaculture in arid Patagonia is potentially affecting the hooded grebe (*Podiceps gallardoi*), a critically endangered endemic waterbird. Exotic rainbow trout (*Oncorhynchus mykiss*) were stocked from 1994 in naturally fishless lakes, the primary reproductive habitat of this grebe.

2. Trout and grebes are visual predators, whose diets overlap. Consequently, trout could reduce the abundance of prey of the hooded grebe.

3. This study compared the size distribution and abundance of the pelagic zooplankton fraction preyed upon by trout in four fishless lakes and three lakes stocked with trout, including vegetated and unvegetated lakes.

4. The mean size of *Daphnia* spp. was 45% and 35% larger in fishless lakes than in stocked lakes, for unvegetated and vegetated lakes, respectively. *Boeckella* spp. were larger in fishless than in stocked vegetated lakes.

5. Fishless and stocked lakes had highly contrasting biomasses of large pelagic crustaceans. Amphipods were absent from the water column of all stocked lakes analysed, and were abundant in fishless lakes. *Parabroteas sarsi* was absent from the two large unvegetated lakes, stocked with trout.

6. These shifts in the abundance and size spectrum of the zooplankton may reflect competition between trout and hooded grebe, affecting the survival of the latter species.

7. The current conservation status of this rare aquatic bird demands the application of management tools to reduce the detrimental effects of aquaculture on their primary reproductive habitat.

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## INTRODUCTION

The hooded grebe (*Podiceps gallardoi*), a diving waterbird endemic to southern Patagonia, lives in cold lakes of this region (Fjeldså, 1984). It breeds in only a few remote basaltic plateaux of Santa Cruz Province, Argentina (Lancelotti *et al.*, 2009a; Roesler *et al.*, 2012). These plateaux collectively hold more than a thousand fishless lakes, including the largest lakes of this arid region. The hooded grebe population was estimated to be 800 individuals in 2010, more than an 80% decline from estimates in the 1980s (Roesler *et al.*, 2012), making it one of the more threatened species in Argentina (Chebez, 2008), as well as globally (Birdlife International, 2015).

More than 40 naturally fishless lakes of the basaltic plateaux were stocked with rainbow trout (*Oncorhynchus mykiss*) for aquaculture from 1994 (Lancelotti *et al.*, 2010b). This has generated concerns about potential adverse effects on the grebe's habitat (Roesler *et al.*, 2011). The introduction of trout was proposed as one of the main causes for the declining grebe population (Roesler *et al.*, 2012). Aquaculture is largely concentrated in Strobel Lake plateau, the historic core of the reproductive habitat of hooded grebe (Fjeldså, 1986; Beltrán *et al.*, 1992). This plateau experienced the most drastic decline in the abundance of hooded grebe (Roesler *et al.*, 2012).

Two breeding lakes for hooded grebe in the Strobel Lake plateau were stocked. Islote Lake (>7 km<sup>2</sup>) was the most important with more than 1000 mature individuals (20–30% of the estimated global population) in a single colony (Beltrán *et al.*, 1992). After fish stocking in 2003, a maximum of 16 mature individuals and no breeding colony were recorded (personal observation). In Ocho Lake (0.2 km<sup>2</sup>), a colony of 81 individuals (40 nests) bred in 2004 (Lancelotti, 2009), but no colonies were recorded 4 years after trout introduction (personal observation). Trout may be reducing habitat suitability for hooded grebe.

Introduced fish have changed the composition and abundance of species, nutrient availability, nutrient cycling, primary productivity, trophic relationships, and behaviour and spatial distribution of prey

species in freshwater ecosystems around the world (Mallory *et al.*, 1994; Scheffer, 1998; Knapp and Matthews, 2001; Schindler and Parker, 2001). Such effects are most dramatic in lakes devoid of native fish (Mallory *et al.*, 1994; Donald *et al.*, 2001; Vredenburg, 2004), such as lakes of the basaltic plateaux of Patagonia. Fish introductions can initiate trophic cascades (Carpenter and Kitchell, 1996), altering drastically the limnological characteristics of lakes (e.g. the quality and abundance of macrophytes; Lauridsen *et al.*, 1994), and reducing habitat for waterbirds (Hornung and Foote, 2006).

Introduced exotic fish and translocation of native fish in lakes of the Patagonian steppe have caused local extinctions of cladoceran species, reduction of zooplankton size, and community homogenization (Modenutti and Balseiro, 1994; Reissig *et al.*, 2006). In the shallow lake Laguna Blanca, stocked fish caused the local extinction of amphibians, reduced significantly the abundance of crustaceans, increased turbidity, induced a decline of macrophytes, and ultimately caused a drastic diminution of waterbird abundance (Ortubay *et al.*, 2006). Despite this, the direct effects of trout are unclear, with confounding effects of native and exotic introduced fish species. Lakes of the Strobel Lake plateau are naturally fishless, and only rainbow trout were stocked, providing an exceptional opportunity to investigate the trophic role of trout and their effects.

Trout and grebes are visual predators with potentially overlapping diets. They feed on similar-sized species, mostly crustaceans (amphipods, copepods and cladocerans), snails, and insects (coleopterans and chironomids) (Fjeldså, 1986; Lancelotti, 2009). Prey abundance is an important factor determining the success of hooded grebe (Fjeldså, 1986).

Most (>95%) of the trout diet (n>400) is composed of pelagic crustaceans, dominated by amphipods (*Hyalella* spp.), and secondarily cladocerans (*Daphnia* spp.) and copepods (*Boeckella* spp.) (Bandieri, 2011, and Julio Lancelotti unpublished data). Changes in this pelagic community may indicate altered food webs. Trout could affect the quantity of pelagic, benthic, and littoral prey on which hooded grebe rely to survive and reproduce.

The size distribution and abundance of the pelagic zooplankton preyed on by trout in fishless and stocked lakes were compared in the Strobel Lake plateau, indicating alterations to the food webs caused by trout introduction. The results were used to assess the consequences for conservation of the critically endangered hooded grebe.

### STUDY AREA

The Strobel Lake plateau is located in the central-west portion of Santa Cruz province (72° 0' W, 48° 30' S, 700–1200 m.a.s.l.), Argentina (Figure 1). It is one of the biggest basaltic plateaux of Patagonia (2500 km<sup>2</sup>), with 301–1244 lakes during extremely dry and wet periods, respectively, which are important for bird species richness (Imberti, 2005; Lancelotti *et al.*, 2009b). Temperatures range between −9°C in winter and 28°C in summer, with frequent and strong winds throughout the year (mean annual speed 24 km h<sup>-1</sup>; Lancelotti, 2009). Water bodies range from large lakes (7 km<sup>2</sup>; <25 m depth) to small temporary ponds (0.005 km<sup>2</sup>, <1 m depth). Lakes vary in water turbidity, macrophyte cover, and physico-chemical properties (Lancelotti *et al.*, 2009b). There are four main types of lakes (Lancelotti *et al.*, 2009b): turbid lakes have high conductivity (>2000 μS cm<sup>-1</sup>) and

turbidity (Secchi depth <0.5 m), varying in size from 9–27 ha and less than 3 m deep. The three remaining groups all have clear water, but differ in their macrophyte cover, depending on size and bathymetry. Small vegetated lakes (<9 ha and <2 m deep) are fully vegetated, while the other two groups comprise larger lakes (>7 ha and 3–16 m deep), either heavily vegetated (large vegetated lakes (V), 15–30% emergent macrophyte cover), or sparsely vegetated (large unvegetated lakes (U), <15% emergent macrophyte cover).

The aquatic invertebrate communities of Strobel Lake plateau have relatively low species richness. Pelagic invertebrates are represented largely by crustaceans, dominated by calanoid copepods (*Boeckella* and *Parabroteas sarsi*), and cladocerans (mostly *Daphnia* spp.). Eight species of calanoid copepods occur in Strobel Lake plateau, but no more than three species are numerically dominant (Lancelotti, 2009). Benthic organisms are primarily represented by Oligochaeta, chironomid larvae, Hirudinea and Gastropoda, although the last of these are not present in all lakes. The distribution of snails is patchy, and absent from lakes in this study. *Hyalella* represent the only genus of amphipods inhabiting Strobel Lake plateau (Lancelotti, 2009). In vegetated lakes adult and larval coleopterans and larvae and pupae of chironomids are abundant

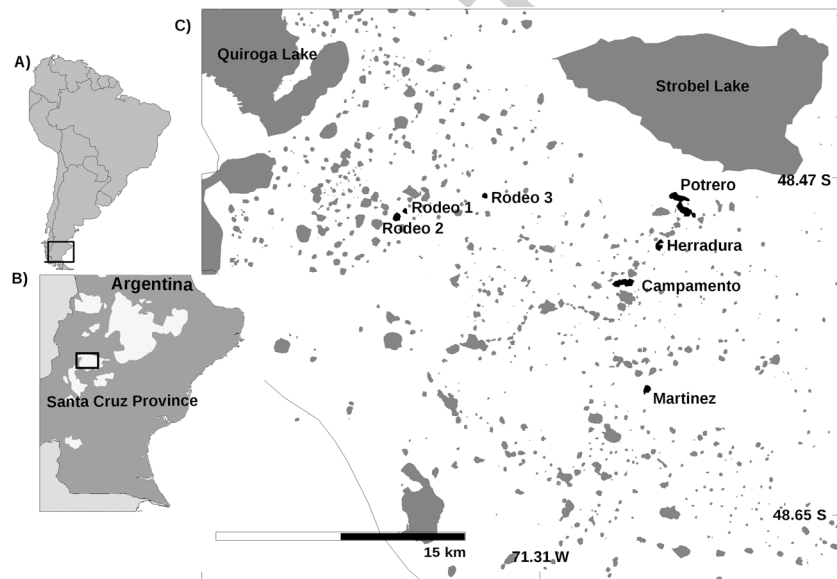


Figure 1. Location of Lake Strobel plateau in (A) Argentina, (B) Santa Cruz Province, and (C) distribution of lakes (grey, classified using Landsat 7 imagery and a land mask), including seven lakes studied (see Table 1 for matching numbers).

among macrophytes. No amphibians or native fish inhabit these lakes. Aquatic vertebrates are only represented by a rich waterbird community, with more than 20 species. Besides hooded grebe, the list of species includes two globally Near Threatened species, the magellanic plover (*Pluvianellus socialis*) and the Chilean flamingo (*Phoenicopterus chilensis*) (Lancelotti *et al.*, 2009b).

Hooded grebe congregate to reproduce in highland lakes from mid-austral spring to early autumn, when they migrate to the Atlantic coast. They use relatively clear and shallow lakes, with a dense carpet of milfoil *Myriophyllum elatinoides*. Nests are built on mat-forming macrophytes, usually surrounded by open water, where grebes forage preferentially on snails, amphipods and insect larvae. These large food items are essential for successfully raising young (Erize, 1983; Fjelds , 1986; Beltr n *et al.*, 1992).

The extensive aquaculture in Strobel Lake plateau consists of stocking fry of rainbow trout (approximately 6000 individuals per km<sup>2</sup>) in medium sized lakes (0.05–0.71 km<sup>2</sup>, mean 0.19 km<sup>2</sup>), and harvesting the fish after 2–3 years, when they reach commercial size (fork length >20 cm). The harvested lakes are then re-stocked. Farmers prefer to stock large unvegetated lakes, where harvesting using gillnets is the easiest fishing method. However, in several of the large vegetated lakes, trout were also stocked. Small vegetated and turbid lakes remain fishless (Lancelotti *et al.*, 2010a). Because of habitat restrictions (i.e. lack of permanent streams and adequate spawning beds), trout do not reproduce in most of the lakes of Strobel Lake plateau; therefore, trout populations are regulated by stocking and harvesting (Lancelotti *et al.*, 2010b).

## METHODS

Zooplankton samples were collected in seven lakes: two fishless and two stocked, large, unvegetated lakes in March 2013, and two fishless lakes and one stocked, large, vegetated lake in December 2014 (Table 1). Three vertical tows of the whole water column were made in each lake, with a 115 µm mesh zooplankton net. This mesh size captures organisms that match the prey size of trout and hooded grebe. Samples were fixed *in situ* with 4% formalin, and analysed in the laboratory with a stereoscopic microscope. Organisms were classified into four categories: *Boeckella* spp. (strongly dominated by *Boeckella poppei* and including *Boeckella michaelsoni*), *Parabroteas sarsi*, *Daphnia* spp. (*Daphnia dadayana*, *Daphnia commutata* and *Daphnia obtusa* group), and *Hyaella* spp. Three subsamples of 20 mL were analysed for each sample by counting all individuals in the four categories.

The body length of 40 randomly selected *Daphnia* spp. was measured (distance from the top of the head to the base of the caudal spine) and *Boeckella* spp. (distance from the base of the first antennae to the tip of the furca). These two groups were present in all lakes, while amphipods and large copepods were absent in several stocked lakes and so were not measured. The size of *Boeckella* spp. and *Daphnia* spp. were compared between fishless and stocked lakes using MANOVA analysis. A square-root transformation was applied, normalizing data distributions, reflected in quantile–quantile plots (Timm, 2002). A nested model design was used to test the effect of individual lakes and status (fishless vs. stocked); interactions were not tested. Large vegetated lakes and large unvegetated lakes were similarly but separately analysed.

Table 1. Status, type (V – large vegetated, U – large unvegetated), area, maximum depth and water quality (conductivity and pH (mean ± (SD)) of seven lakes in the Lake Strobel plateau (updated from Lancelotti, 2009 and Lancelotti *et al.*, 2009a). Lake locations are shown in Figure 1

Lake	Status*	Type	Area (km <sup>2</sup> )	Max depth (m)	Conductivity (µs cm <sup>-1</sup> )	pH
Rodeo 1	Fishless	U	0.076	5	169 (1.2)	8.7 (0.1)
Rodeo 2	Fishless	U	0.184	5	173 (47)	8.6 (0.1)
Rodeo 3	Fishless	V	0.077	NA	186 (NA)	8.4 (NA)
Martinez	Fishless	V	0.153	12	294 (25)	9 (1.0)
Campamento	1996	U	0.428	16	87 (14)	8.9 (1.0)
Potrero	2001	U	0.786	15	312 (30)	9.8 (1.2)
Herradura	2000	V	0.185	9	127 (30.5)	9.9 (1.1)

\*Date of the first fish stocking

The biomass ( $\text{mg L}^{-1}$ ) of each category of organism was estimated by multiplying its abundance in samples by the mean individual mass from previous studies in the same lake system (Lancelotti, 2009), and length–weight relationships from literature (Pilati and Martínez, 2003). A principal component analysis (PCA) (Legendre and Legendre, 2003) tested the similarity of community assemblages between lakes. Variables included in the covariance matrix for the PCA were the biomass ( $\text{g m}^{-3}$ ) of *Boeckella* spp., *Daphnia* spp., *P. sarsi*, and *Hyaella* spp. Data were normalized with a square-root transformation, and all variables were standardized. Statistical analyses were conducted with R software (R Development Core Team, 2013).

## RESULTS

There were significant differences in size of *Boeckella* spp. among large unvegetated lakes, although this was not related to whether they were fishless or stocked (Table 2, Figure 2), which contrasted fishless and stocked vegetated lakes with significantly different abundances of *Boeckella* spp. The opposite results were observed in large vegetated lakes for this group, with significant differences between fishless and stocked lakes, and no significant differences at the level of individual lakes. *Daphnia* spp. were consistently larger in fishless than in stocked vegetated and

Table 2. Summary of the Manova analysis, comparing the body size of *Boeckella* spp. and *Daphnia* spp. between fishless and stocked lakes. Vegetated (V) and unvegetated (U) lakes were sampled in different periods and were analysed separately. ‘Status’ refers to fishless or stocked, and ‘Lake’ to the effect of individual lakes

Lake type	Variable	DF	F value	P-value
		<i>Boeckella</i> spp.		
V	Status	1	0.3	0.61
	Lake	2	108.1	<0.001
U	Status	1	60	<0.001
	Lake	2	0.1	0.76
		<i>Daphnia</i> spp.		
V	Status	1	178.6	<0.001
	Lake	2	9.3	<0.001
U	Status	1	80.6	<0.001
	Lake	2	0.4	0.51

unvegetated lakes, although there were differences among lakes (Table 2, Figure 2). *Daphnia* spp. were 45% and 35% larger in fishless lakes than in stocked lakes, respectively, for unvegetated and vegetated lakes (Figure 2).

Fishless and stocked lakes showed highly contrasting biomasses of large pelagic crustaceans (>1 mm; Figure 3). The most remarkable difference was the absence of *Hyaella* spp. in the water column of every stocked lake, and *Parabroteas* in the two large unvegetated stocked lakes. In contrast, these organisms contributed substantially to the biomass in fishless lakes (but *Hyaella* spp. in Rodeo 3). These differences in biomass between fishless and stocked lakes were not evident in the groups of smaller body size – *Daphnia* spp. and *Boeckella* spp. Their biomass was more variable among individual lakes than between fishless and stocked lakes or lake type (vegetated vs. unvegetated). For example, the stocked lake Potrero had similar biomass of *Daphnia* spp. to the two fishless lakes, whereas the stocked lake Campamento had similar biomass of *Boeckella* spp. to these fishless lakes (Figure 3). Moreover, the vegetated lakes, Rodeo 3, and Herradura (fishless and stocked respectively; Figure 3, lower panel), had similar biomass of *Daphnia* spp. and *Boeckella* spp.

With respect to community assemblages, *Boeckella* spp. explained most of the variability in the first principal component, whereas *P. sarsi* and *Hyaella* spp. explained most of the variability in the second principal component (Figure 4, Table 3). Fishless and stocked lakes were arranged in separate groups, explained mostly by the biomass of *P. sarsi* and *Hyaella* spp. The two large, fishless unvegetated lakes (Rodeo 1 and Rodeo 2) had high biomass of *P. sarsi* and *Hyaella* spp., respectively. However, Rodeo 1 also had a high abundance of *Boeckella* spp. The two large unvegetated stocked lakes (Potrero and Campamento) showed the opposite in terms of large crustacean biomass, whereas there was no clear pattern in the large vegetated lakes. The stocked Herradura Lake, characterized by its low crustacean biomass, represented the most atypical lake, with a community structure similar to the fishless vegetated lake Rodeo 3.

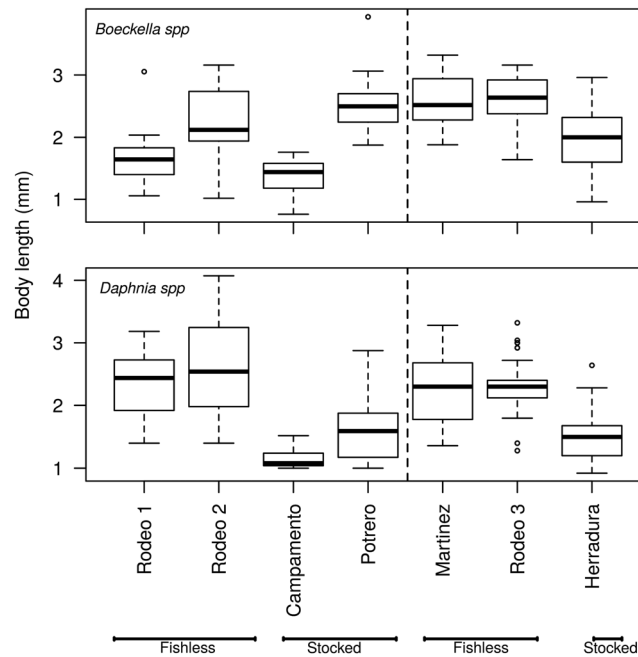


Figure 2. Body length of *Boeckella* spp. (upper panel) and *Daphnia* spp. (lower panel) collected in four fishless lakes (Rodeo 1, Rodeo 2, Martínez, and Rodeo 3), and three stocked lakes (Potrero, Campamento and Herradura) of the Lake Strobel plateau. The dotted line separates large unvegetated lakes (left) from large vegetated lakes (right). Horizontal lines represent median values of body size. Lower and upper hinges represent 25% and 75% percentiles, respectively.

## DISCUSSION

Rainbow trout shaped the community of pelagic crustaceans, affecting species' dominance and size structure. The most evident and drastic effect was the absence of large crustaceans (*P. sarsi* and *Hyalella* spp.) in stocked lakes. These probably represent the primary prey item of hooded grebes, affecting breeding success of this endangered species. The observed differences reflect trout predation. During early stages of aquaculture, amphipods represented more than 95% of the stomach content of rainbow trout, but their relative importance has decreased over time (Bandieri, 2011). *Daphnia* spp., which were occasionally preyed upon by trout during the early phases of aquaculture, became the primary prey after two or more cycles of trout stocking (Bandieri, 2011).

Trout also exert impacts on benthic and littoral communities by feeding on large coleopterans, chironomid larvae, and snails in the littoral zone. For example, chironomid larvae were the primary prey for trout where the abundance of crustaceans was low (Lancelotti *et al.*, 2015). Furthermore, in Cardiel Lake (300 km<sup>2</sup>), at the base of the Strobel

Lake plateau, trout prey primarily on snails. This lake is depleted of crustaceans, probably as a direct effect of trout predation (Pascual *et al.*, 2001). These changes in benthic and littoral communities may also be occurring in the Strobel Lake plateau.

The decrease in prey size and biomass may also affect bioenergetic costs, reproductive success, and fitness of the grebe. Additionally, zooplankton grazing pressure on phytoplankton may be reduced by trout, with increasing nutrient recycling, promoting phytoplankton growth and potentially causing trophic cascades (Carpenter and Kitchell, 1996; Eby *et al.*, 2006). A recent study in the Lake Strobel plateau found that stocked lakes have substantially higher levels of Cyanobacteria than fishless lakes (Izaguirre and Saad, 2014), indicating the occurrence of changes in the phytoplankton communities.

These processes may be more serious in vegetated lakes, where the abundance and quality of macrophytes can be affected, altering the suitability of these lakes as reproductive habitat for hooded grebes. Although aquaculture is concentrated in large unvegetated lakes (Lancelotti *et al.*, 2010b), several large vegetated lakes were stocked. They are

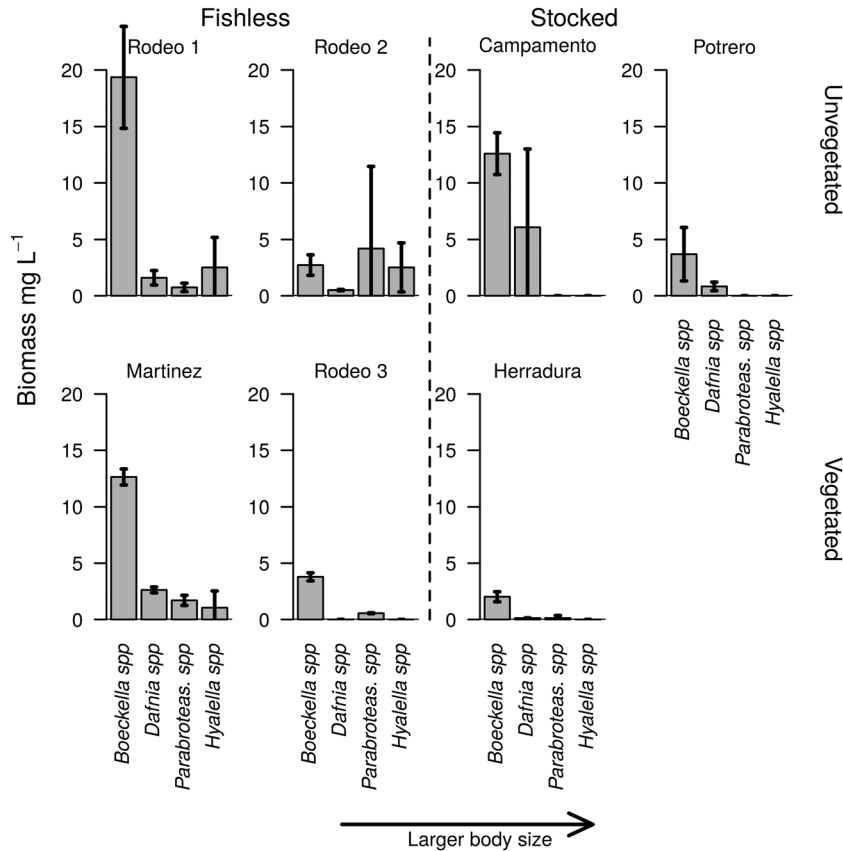


Figure 3. Mean ( $\pm$  sd, n=3) biomass of pelagic crustaceans in seven lakes of the Lake Strobel plateau. The upper and lower panels correspond respectively to large unvegetated and large vegetated lakes. The dashed line separates fishless (left panel) and stocked (right panel) lakes.

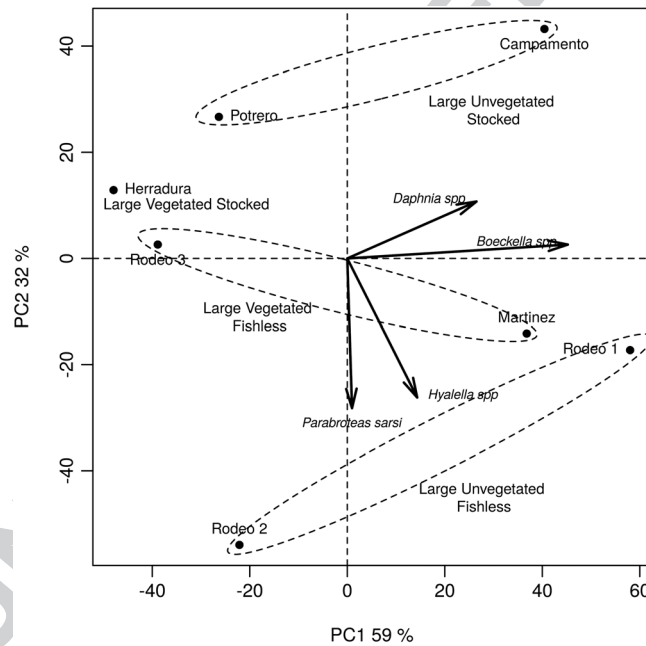


Figure 4. Ordination of lakes on the two first principal components (PC), explaining 91% of variation in biomass of different pelagic crustacean groups, with arrows representing their relative weight. Ovals identify (visually) lake types (large vegetated vs. large unvegetated) and status (fishless vs. stocked).

Table 3. Summary of the eigenvectors of the principal component analysis (PCA) of different species separating the seven lakes

Variable	PC 1	PC 2	PC 3
<i>Boeckella</i> spp.	0.83	0.06	0.49
<i>Daphnia</i> spp.	0.49	0.27	-0.83
<i>Parabroteas sarsi</i>	0.02	-0.70	-0.27
<i>Hyaella</i> spp.	0.26	-0.65	0.01
Variance explained (%)	59	32	7

likely to be used increasingly as the preferred non-vegetated lakes are stocked. This may exacerbate the spatial overlap between trout and hooded grebes (Lancelotti *et al.*, 2010b).

Grebe species are particularly vulnerable to introductions of exotic fish. The introduction of fishes (mostly rainbow trout) threatens seven of the nine species of grebes of conservation concern listed by the IUCN (<http://www.iucnredlist.org/>), including two recently extinct species (atitlán grebe *Podilymbus gigas* and andean grebe *Podiceps andinus*). In addition, gill nets used for fishing are among the main causes of grebe deaths worldwide (Fjeldså, 2004). Gill nets used in the Lake Strobel area are potential further threats to hooded grebes although little is known of their impact on mortality.

The critical status of hooded grebe demands actions focused on reducing the overlap between aquaculture and the habitat of this species. In 2014 the Patagonia National Park was created to protect primary reproductive areas of hooded grebes. However, habitats suitable for hooded grebe reproduction need to be conserved in their original fishless condition. It is also imperative to prevent fish harvesting overlapping with the presence of hooded grebe. There is an opportunity, too, to rehabilitate reproductive areas of hooded grebes, invaded by trout, by removing these fish and returning the lakes to a fishless status. These actions together could save this endangered species from the impacts of trout.

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#### REFERENCES

- Bandieri L. 2011. *Dieta y crecimiento de la trucha arco iris (Oncorhynchus mykiss) en lagunas de la meseta del Lago Strobel, Santa Cruz*. Universidad Nacional de la Patagonia.
- Beltrán J, Bertonatti C, Johnson A, Serret A, Sutton P. 1992. Actualizaciones sobre la distribución, biología y estado de conservación del Macá tobiano (*Podiceps gallardoi*). *El Hornero* **13**: 193–209.
- Birdlife International. 2015. Species factsheet: *Podiceps gallardoi*. IUCN Red List for birds. Downloaded from <http://www.birdlife.org> on 18/02/2015.
- Carpenter SR, Kitchell JF. 1996. *The Trophic Cascades in Lakes*. Cambridge University Press: Melbourne.
- Chebez JC. 2008. *Los que se van. Fauna argentina amenazada*. Albatros: Buenos Aires.
- Donald DB, Vinebrooke RD, Anderson RS, Syrgiannis J, Graham MD. 2001. Recovery of zooplankton assemblages in mountain lakes from the effects of introduced sport fish. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 1822–1830.
- Eby LA, Roach WJ, Crowder LB, Stanford JA. 2006. Effects of stocking-up freshwater food webs. *Trends in Ecology and Evolution* **21**: 576–584.
- Erize F. 1983. Observaciones sobre el Macá Tobiano. 2006. *El Hornero* **12**(extra): 256–68.
- Fjeldså J. 1984. Three endangered South American grebes (*Podiceps*): case histories and the ethics of saving species by human intervention. *Annales Zoologici Fennici* **21**: 411–416.
- Fjeldså J. 1986. Feeding ecology and possible life history tactics of the hooded grebe *Podiceps gallardoi*. *Ardea* **74**: 40–58.
- Fjeldså J. 2004. *The Grebes*. Oxford University Press: New York.
- Hornung J, Foote A. 2006. Aquatic invertebrate responses to fish presence and vegetation complexity in western boreal wetlands, with implications for waterbird productivity. *Wetlands* **26**: 1–12.
- Imberti S. 2005. Meseta Lago Strobel. In *Áreas Importantes para la Conservación de las Aves en Argentina. Sitios Prioritarios para la Conservación de la Biodiversidad*, Di Giacomo AS (ed). Aves Argentinas/Asociación Ornitológica del Plata: Buenos Aires; 415–416.
- Izaguirre I, Saad JF. 2014. Phytoplankton from natural water bodies of the Patagonian Plateau. *Advances in Limnology* **65**: 309–319.
- Knapp RA, Matthews KR. 2001. Resistance and resilience of alpine lake fauna to fish introductions. *Ecological Monographs* **71**: 401–421.



- Lancelotti JL. 2009. *Caracterización limnológica de lagunas de la Provincia de Santa Cruz y efectos de la introducción de Trucha Arco iris (Oncorhynchus mykiss) sobre las comunidades receptoras*. Universidad Nacional del Comahue: Río Negro, Argentina.
- Lancelotti JL, Pozzi LM, Yorio PM, Diéguez MC, Pascual MA. 2009a. Fishless shallow lakes of Southern Patagonia as habitat for waterbirds at the onset of trout aquaculture. *Aquatic Conservation: Marine and Freshwater Ecosystems* **19**: 497–505.
- Lancelotti JL, Pozzi LM, Márquez F, Yorio PM, Pascual MA. 2009b. Waterbird occurrence and abundance in the Strobel Plateau, Patagonia, Argentina. *Hornero* **24**: 13–20.
- Lancelotti JL, Pascual MA, Gagliardini A. 2010a. A dynamic perspective of shallow lakes of arid Patagonia as habitat for waterbirds. In *Ponds: Formation Characteristics and Uses*, Meyer PL (ed). Nova Science Publishers: New York.
- Lancelotti JL, Pozzi LM, Yorio PM, Diéguez MC, Pascual MA. 2010b. Precautionary rules for exotic trout aquaculture in fishless shallow lakes of Patagonia: minimizing impacts on the threatened hooded grebe (*Podiceps gallardoi*). *Aquatic Conservation: Marine and Freshwater Ecosystems* **8**: 1–8.
- Lancelotti JL, Bandieri LMB, Pascual MA. 2015. Diet of the exotic rainbow trout in the critical habitat of the threatened hooded grebe. *Knowledge and Management of Aquatic Ecosystems* **416**: 1–11.
- Lauridsen TL, Jeppesen E, Søndergaard M. 1994. Colonization and succession of submerged macrophytes in shallow Lake Væng during the first five years following fish manipulation. *Hydrobiologia* **275–276**: 233–242.
- Legendre P, Legendre L. 2003. *Numerical Ecology*. Elsevier: New York.
- Mallory ML, Blancher PJ, Weatherhead PJ, McNicol DK. 1994. Presence or absence of fish as a cue to macroinvertebrate abundance in boreal wetlands. *Hydrobiologia* **279–280**: 345–351.
- Modenutti BE, Balseiro EG. 1994. Zooplankton size spectrum in four lakes of the Patagonian Plateau. *Ecology Letters* **11**: 470–480.
- Ortubay S, Cussac VE, Battini M, Barriga J, Aigo J, Alonso M, Macchi P, Reissig M, Yoshioka J, Fox SF. 2006. Is the decline of birds and amphibians in a steppe lake of northern Patagonia a consequence of limnological changes following fish introduction? *Aquatic Conservation: Marine and Freshwater Ecosystems* **16**: 93–105.
- Pascual MA, Frere E, Arguimbau M, Pellanda L. 2001. *Los recursos pesqueros del Lago Cardiel: diagnóstico y sugerencias para su manejo*. Universidad Nacional de la Patagonia Austral, Unidad Académica Caleta Olivia.
- Pilati A, Martínez J. 2003. Relación longitud-peso de siete especies de *Boeckella* (Copepoda: Calanoida) de la República Argentina. *Neotrópica* **55–61**.
- R Development Core Team. 2013. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. URL <http://www.R-project.org/>.
- Reissig M, Trochine C, Queimalinos C, Balseiro E, Modenutti BE. 2006. Impact of fish introduction on planktonic food webs in lakes of the Patagonian Plateau. *Biological Conservation* **132**: 437–447.
- Roesler I, Casañas H, Imberti S. 2011. Final countdown for the hooded grebe? *Neotropical Birding* **9**: 3–7.
- Roesler I, Imberti S, Casañas H, Mahler B, Reboreda JC. 2012. Hooded grebe *Podiceps gallardoi* population decreased by eighty per cent in the last twenty-five years. *Bird Conservation International* **22**: 371–382.
- Scheffer M. 1998. *Ecology of Shallow Lakes*. Chapman & Hall: New York.
- Schindler DW, Parker BR. 2001. Biological pollutants: alien fishes in mountain lakes. *Water Air and Soil Pollution: Focus* **2**: 379–397.
- Timm NH. 2002. *Applied Multivariate Analysis*. Springer New York: New York.
- Vredenburg VT. 2004. Reversing introduced species effect: experimental removal of introduced fish leads to rapid recovery of a declining frog. *Proceedings of the National Academy of Sciences of the United States of America* **36**: 7646–7650.

# Author Query Form

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**Journal: Aquatic Conservation: Marine and Freshwater Ecosystems**

**Article: aqc\_2629**

Dear Author,

During the copyediting of your paper, the following queries arose. Please respond to these by annotating your proofs with the necessary changes/additions.

- If you intend to annotate your proof electronically, please refer to the E-annotation guidelines.
- If you intend to annotate your proof by means of hard-copy mark-up, please use the standard proofing marks. If manually writing corrections on your proof and returning it by fax, do not write too close to the edge of the paper. Please remember that illegible mark-ups may delay publication.

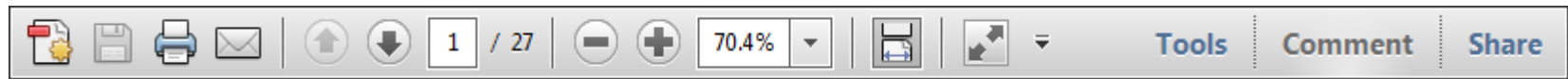
Whether you opt for hard-copy or electronic annotation of your proofs, we recommend that you provide additional clarification of answers to queries by entering your answers on the query sheet, in addition to the text mark-up.

Query No.	Query	Remark
Q1	AUTHOR: Please confirm that given names (red) and surnames/family names (green) have been identified correctly.	
Q2	AUTHOR: Use of term “hinges” not understood. Please correct or confirm.	

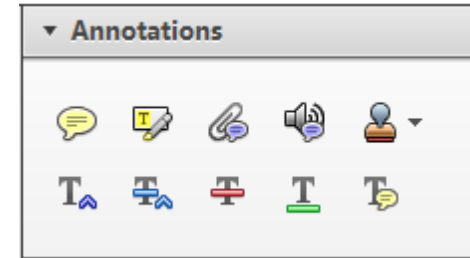
Required software to e-annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 7.0 or above). (Note that this document uses screenshots from Adobe Reader X)

The latest version of Acrobat Reader can be downloaded for free at: <http://get.adobe.com/uk/reader/>

Once you have Acrobat Reader open on your computer, click on the [Comment](#) tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the [Annotations](#) section, pictured opposite. We've picked out some of these tools below:



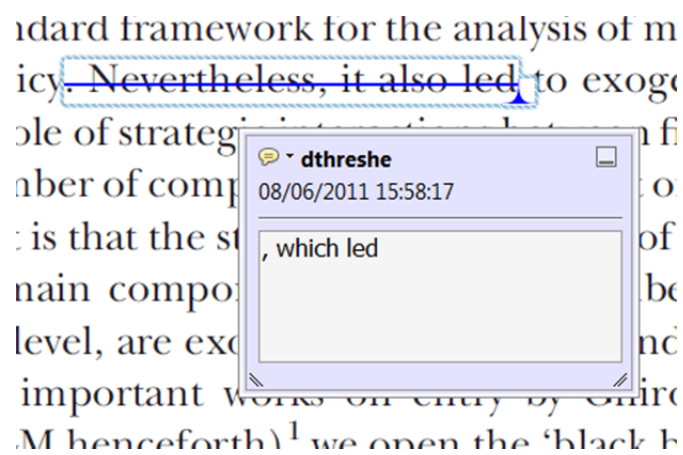
**1. Replace (Ins) Tool – for replacing text.**



Strikes a line through text and opens up a text box where replacement text can be entered.

**How to use it**

- Highlight a word or sentence.
- Click on the [Replace \(Ins\)](#) icon in the Annotations section.
- Type the replacement text into the blue box that appears.



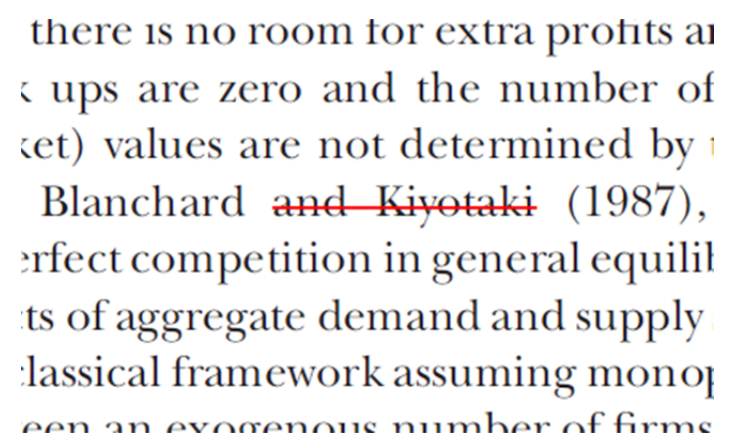
**2. Strikethrough (Del) Tool – for deleting text.**



Strikes a red line through text that is to be deleted.

**How to use it**

- Highlight a word or sentence.
- Click on the [Strikethrough \(Del\)](#) icon in the Annotations section.



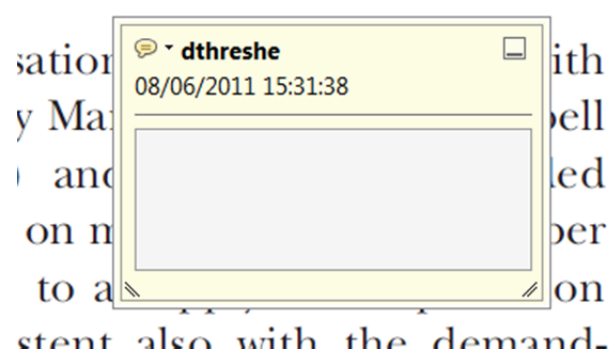
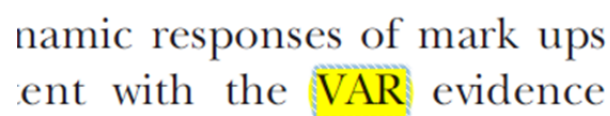
**3. Add note to text Tool – for highlighting a section to be changed to bold or italic.**



Highlights text in yellow and opens up a text box where comments can be entered.

**How to use it**

- Highlight the relevant section of text.
- Click on the [Add note to text](#) icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.



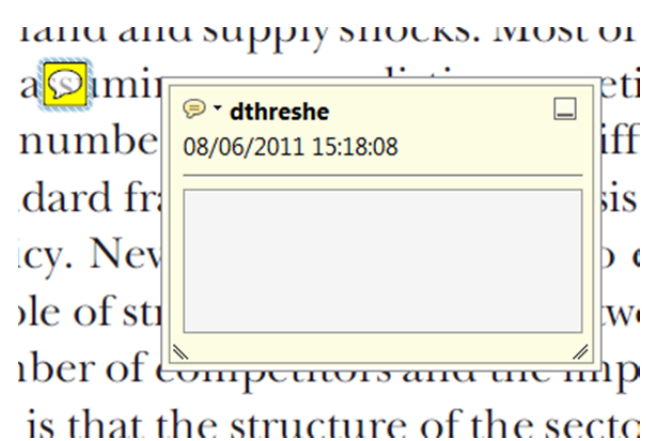
**4. Add sticky note Tool – for making notes at specific points in the text.**



Marks a point in the proof where a comment needs to be highlighted.

**How to use it**

- Click on the [Add sticky note](#) icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



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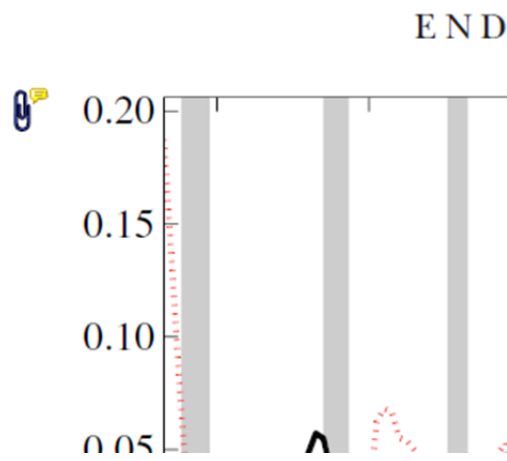
**5. Attach File Tool – for inserting large amounts of text or replacement figures.**



Inserts an icon linking to the attached file in the appropriate place in the text.

**How to use it**

- Click on the [Attach File](#) icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



**6. Add stamp Tool – for approving a proof if no corrections are required.**

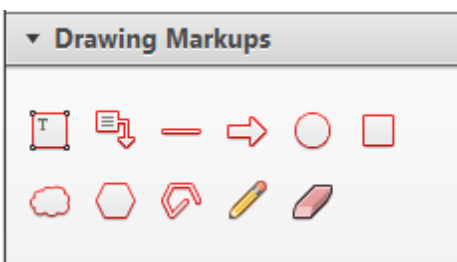


Inserts a selected stamp onto an appropriate place in the proof.

**How to use it**

- Click on the [Add stamp](#) icon in the Annotations section.
- Select the stamp you want to use. (The [Approved](#) stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

of the business cycle, starting with the  
 on perfect competition, constant return  
 production. In this environment goods  
 extra profits and the number of firms  
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 otaki (1987), has introduced product  
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 ed and supply shocks. Most of this literat

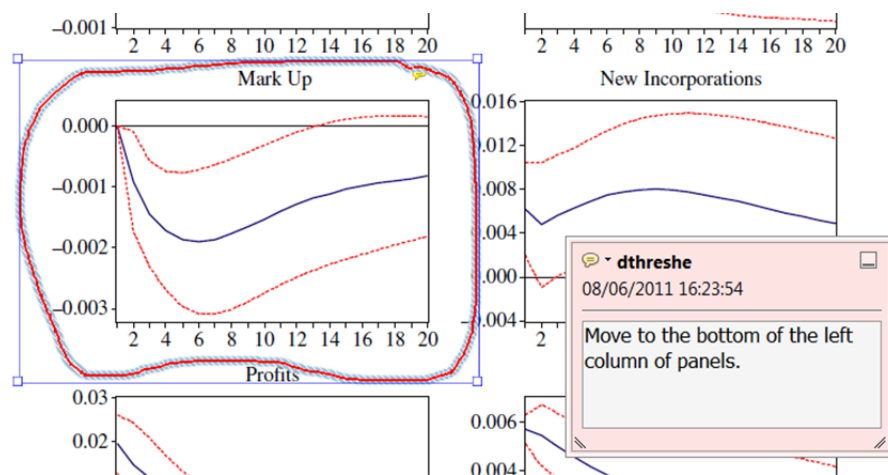


**7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.**

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

**How to use it**

- Click on one of the shapes in the [Drawing Markups](#) section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the [Help](#) menu to reveal a list of further options:

