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Filter feeding by the freshwater mussel, *Diplodon chilensis*, as a biocontrol of salmon farming eutrophication

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Abstract

The freshwater mussel *Diplodon chilensis* (Hyriidae) is abundant in bays where salmon farming takes place in southern Chilean lakes. In order to evaluate the possibility of mitigating salmon farming impacts through the management of benthic communities, we measured the ability of *D. chilensis* to filter algae and clear the water column of particulates and dissolved nutrients associated with salmon farms. A 3 month experiment growing juvenile salmon with and without mussels was conducted in outdoor tanks where dissolved nutrients and chlorophyll *a* were measured before, during and after addition of mussels. In addition, the filtering ability of mussels was measured in laboratory aquaria under different algal concentrations ranging from oligotrophic to hypereutrophic. Within 18 days, *D. chilensis* reduced chlorophyll *a* concentrations in tanks with fish by two orders of magnitude (from ~ 300 to 3 $\mu g\ l^{-1}$) compared to tanks without it. Concentrations of total phosphorous, PO_4 and NH_4 were also reduced by about one order of magnitude after 18 days, through to day 39 from the beginning of the experiment. Thus, mussels were able to change a hypereutrophic situation resulting from salmon culture to an oligotrophic one. Since the tanks were closed systems, the effect of mussels declined by day 61, probably due to the excessive accumulation of organic matter. In the aquarium experiments, *D. chilensis* showed a maximum cell retention rate (60×10^6 cells $ind^{-1}\ h^{-1}$) at chlorophyll concentrations between 20 and 30 $\mu g\ l^{-1}$. Considering their high filtering rate (ca. 1.31 $h^{-1}\ ind^{-1}$) and high density in Chilean lakes, particularly in coastal areas and bays (50 to 200 ind m^{-2}), mussels may exert a considerable filtering effect on lakes. In addition, mussels may play an important role in reducing nutrient loadings.

Keywords: Filter-feeding; Mitigation; Salmon; Freshwater mussel; Eutrophication

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1. Introduction

1.1. Bivalve impacts as filter feeders in estuaries and bays

It is well known that bivalves, particularly mussels, are efficient filter feeders capable of depleting the water column of phytoplankton (Dame et al., 1985, 1991). It is also clear that mussels play a very important role in the coupling between the water column and the benthos in coastal areas and estuaries (Cloern, 1982). These bivalves not only deplete the plankton from the water column, but they also accumulate nutrients and thereby change their absolute and relative concentration both in the sediments and in the water column. Filter feeders could have a great impact on phytoplankton nutrient limitation, as suggested by Elser et al. (1996). Indeed bivalves could be considered key species when they behave as the most efficient filter-feeders in a particular system. For example, Prins and Smaal (1994) demonstrated that *Mytilus edulis* contributed strongly to N remineralization and retained little N in the mussel beds, but it captured proportionally more P.

Although most published work on filter feeding has focused on marine bivalves, some work has been done with freshwater bivalves, particularly *Dreissena polymorpha.* This species was shown to be an efficient filter feeder in European (Reeders et al., 1989) and North American lakes (Bunt et al., 1993) where water transparency increases and chlorophyll decreases were reported after its introduction. Thus, the species has been proposed as a top–down grazer appropriate for biomanipulation (Carpenter and Kitchell, 1993). The main problems with this mussel, however, are its ability to foul all kinds of submerged aquatic structures and its fast spreading ability, which make it a problematic invasive species (Hebert et al., 1991; Griffiths et al., 1991).

1.2. Salmon culturing in floating pens and their impact

Salmon farming utilizing floating pens increases N and P inputs to the lake, bay or estuary where the pens are located (Ackefors and Enell, 1990). These additional nutrient loads can generate and enhance eutrophication. Many Baltic countries have diminished the nutrient load produced by salmon farms in order to reduce the associated eutrophication problem and algae blooms (Makinen, 1991). So far, most solutions have involved diminishing the P concentration in diets rather than finding other ways to handle the excess nutrients that are left in the aquatic environments.

Salmon farming in Chile has increased exponentially during the past 10 years, making it the second largest producer in the world. Total exports during 1995 reached 100,000 tonnes. The most commonly farmed species are *Oncorhynchus mykiss*, *O. kisutch* and *Salmo salar*.

One of the reasons for salmon farming success in Chile has been the water quality in the farming areas, particularly the lakes, which are deep, oligotrophic and very transparent (Soto and Stockner, 1996). However, this quality may change since salmon farming in floating cages adds nutrients to the benthos and water column. It has been calculated that approximately 70% of the P and 30–50% of N in the salmon feed is unassimilated

Table 1
Some water quality parameters of Lake Llanquihue (41°08′S, 72°47′W), southern Chile

Site	Secchi transparency (m)	Total P ($\mu g\ l^{-1}$)	Chl *a* ($\mu g\ l^{-1}$)
Totoral Bay	12.4 (0.9)	12.5 (4.8)	1.9 (0.3)
Frutillar Bay	14.2 (0.6)	6.9 (2.6)	1.3 (0.2)
Center of Lake	19.0 (0.8)	4.2 (0.8)	1.0 (0.2)

Values shown are average values (± s.e.) for the epilimnion during spring and summer. Chl *a*, Chlorophyll *a*; P, Phosphorus.

by the fish and released to the environment (Beveridge, 1996). Eutrophication associated with salmon farming may be severe in lakes, particularly if the renewal time is long.

Lake Llanquihue (42°S, 72°W) is the largest (840 km^2 and 340 m maximum depth) lake in Chile. More than 80% of the salmon smolt in the country are farmed in the lake, producing approximately 2500 tonnes of fish per year. This level of fish production results in the addition of approximately 40 tonnes of phosphorus, or 13% of the lake's total supply (Soto and Campos, 1995). Despite this high load, salmon farming bays still look relatively pristine, and water chlorophyll content is still low (Table 1). Soto and Stockner (1996) suggested some mechanisms to explain the apparent resistance of lakes to nutrient inputs. However, at that time, the authors did not consider the contribution of filter-feeder bivalves to water clarity since little was known.

In the coastal areas and bays of Lake Llanquihue, there are large, dense beds of freshwater mussels, *Diplodon chilensis chilensis* (Gray, 1828), Hyriidae, in soft bottom substrates. This bivalve, native of Chilean and Argentinean freshwater habitats, grows to a large size (8 cm shell length), is long-lived, slow growing, and achieves very high densities in areas with high organic inputs (Parada et al., 1989). Considering the importance of filter feeding bivalves to the pelagic–benthic nutrient coupling in other systems, and the high density of *Diplodon* in lake Llanquihue, this bivalve may affect nutrient inputs associated with salmon farming in the lake. Thus, it may be possible to mitigate adverse effects of nutrient inputs by enhancing or manipulating mussel densities. One advantage of using *Diplodon* for this purpose is that, unlike *Dreissena*, this species occurs naturally in Chilean lakes and does not produce the fouling problems associated with other bivalves.

The purpose of this study was to evaluate the filter feeding ability and impact of *D. chilensis* on experimental aquatic ecosystems under the influence of salmon culture. To do so, we performed manipulative experiments with salmon and *Diplodon* in outdoor tanks and in aquaria. To extrapolate results from these experiments, we evaluated the mussel density in Totoral Bay, located in Lake Llanquihue, which contains intensive salmon farming.

2. Methods

2.1. Outdoor experiments

To demonstrate the cleaning effect of *D. chilensis* on a water column impacted by salmon farming, we performed a 3 month experiment in twelve 450 l fiberglass tanks

(94 cm diameter, 110 cm high). These tanks were located outdoors in a small stream fed from a cold groundwater spring. Each tank was submerged up to 25 cm from the bottom to minimize daily temperature changes because the tanks were exposed to sunlight and rain. The tanks had a small overflow device with netting near the upper border to avoid overflow when it rained. The experimental site is located inside the University Campus, about 30 km south of Lake Llanquihue, and all mussels used in the experiments were freshly collected from this lake.

In November 1994, the tanks were filled with spring water and 50 kg of clean sand from Lake Llanquihue was added to each tank to serve as substrate for the mussels. On November 24, we added 35 specimens of *D. chilensis* to each of 6 tanks. They were chosen by size classes most represented in the lake: five individuals in the 3^+ (3.0–3.9) cm class and 10 individuals in each of the 4^+, 5^+ and 6^+ cm size classes. The number of mussels per tank was equivalent to the 50 ind m^{-2} density and approximately 25 g (dry biomass) m^{-2} found in Totoral Bay, one of the bays with salmon farming from which the mussels were collected (Mena, 1997).

On November 26 we added 4 juvenile pacific salmon (*O. kisutch*), weighing 4.0 g each, to each of 6 tanks, such that we had a factorial design with two factors: (1) presence or absence of mussels, 'mussel treatment' and (2) presence or absence of salmon, 'salmon treatment'. The tanks with fish received 1.6 g of salmon feed twice a day so that each individual received between 15 and 20% of its weight daily during the experiment. These conditions simulate the densities and growing conditions of commercial salmon in Chilean lake cages. Thus, the 'salmon' treatment included both the fish and its food with associated consequences, such as feed losses, feces and excretion. We did not attempt to separate each of these effects.

Water was not exchanged in the tank during the experiment, except for rain which produced approximately 30% water replacement. There was no oxygenation except for stirring the water slowly twice a day in each tank. These conditions attempted to produce a very eutrophic situation in a short time.

This experiment lasted from mid November until the end of January. Water samples were collected every 20 days by lowering a sampling bottle 50 cm below the water surface for nutrient analysis [Total Phosphorous (TP) and inorganic dissolved nutrients (PO_4, NO_2–N, NO_3–N, NH_4)]. We also measured the concentration of TP and TN (Total Nitrogen) in tank sediments, taking a 200 g sediment sample with a hand-made sediment trap.

In order to measure chlorophyll *a* (Chl*a*), 250 ml of water was filtered through Whatman GF/C filters. Pigments were extracted with methanol and quantified using a Turner 10AU fluorometer after acidification to correct for the presence of phaeopigments (Axler and Owen, 1994).

We measured dissolved oxygen each day with a Jenway portable oxygen-meter by holding the oxygen probe near the bottom of each tank. pH was measured in a similar way with a Jenway portable pH-meter.

A two-way ANOVA was performed on each response variable on each sampling date, 18, 39, and 61 days after the beginning of the experiment. We also conducted paired comparisons (Tukey) between 'control' and 'mussel' treatments, and between 'salmon' and 'salmon–mussel' treatments. These were the only statistically meaningful

comparisons according to the main hypothesis to be tested (Mead, 1988) which is the cleaning effect of *D. chilensis* under salmon farming conditions.

To evaluate the ability of the aquatic system to recover after the proposed 'cleaning' mussel effect, after 61 days all mussels were withdrawn from the three tanks with the salmon–mussel treatment and from the mussel treatment tanks. Also, 35 mussels were added to the three tanks of the salmon farming treatment to determine whether the addition of mussels could mitigate salmon effects. The three control treatment tanks (no mussels nor salmon) remained undisturbed in order to be used as controls for other external variables. Since there were no true replicates for this manipulation (the three tanks of each treatment were disturbed with the elimination or addition of mussels except for the controls there was no further statistical analysis and the raw data for individual tanks are shown graphically. Due to budget restrictions, only Chl*a* measurements were conducted for all tanks on days 83 and 102.

To evaluate the physiological state of the mussels under the experimental conditions, we marked the individuals and measured total shell length and wet weight before and after the experiment. We also measured dry weight at the end. Thus, we calculated a condition index, $K = (P/L^3) \times 100$, where P is the dry weight (soft parts plus shell) in grams, and L is the total shell length in centimeters (Lara and Parada, 1991). To calculate K at the beginning of the experiment we only used wet weight but extrapolated to dry weight based on a previously known correlation.

2.2. Filter feeding experiments

To evaluate *Diplodon* filter feeding rates, particle retention rate and pseudofeces production, several experiments were conducted in the laboratory between May and September 1995. To assess filtration rate and particle retention rates at several algal and Chl*a* concentrations, we performed three experiments. The first experiment used Chl*a* concentrations from 1 to 300 $\mu g\ l^{-1}$, the second used a low-to-moderate algal concentration (1–30 $\mu g\ l^{-1}$ Chl*a*) and the third used a moderate-to-high concentration (20–80 $\mu g\ l^{-1}$ Chl*a*). In each of the them we used six algal concentrations with two replicates per concentration. One individual *D. chilensis* of 50 to 55 mm total shell length (the most representative size in the lake) was maintained for 1 h in a 500-ml aquarium containing microalgae at the required concentration. Aquaria were maintained with air-bubbling and little stirring in order to avoid settling of the algae. There were two controls without mussels for each algal concentration. The algae was obtained from a batch culture which consisted mainly of *Closterium aff. acutum* (65%), *Kirchneriella* sp. (10%), *Anabaenopsis* sp. (1%), *Monoraphydium* sp. (8%), *Scenedesmus* sp. (14%) and *Cuadrigula* sp. (2%). Variation in the species relative abundance was less than 5% among experiments. These species, particularly *Closterium* and *Scenedesmus*, are common in the shallow bays of the lake in summer.

Filtration rate was calculated using Reeders et al. (1989) formula:

$$\text{Filtration rate}\,(1\,h^{-1}) = V/t,[(\ln Ci - \ln Cf) - (\ln C'i - \ln C'f)]$$

where: Ci = Initial cell concentration (cells l^{-1}); Cf = Final cell concentration (cells l^{-1}); t = Experimental time span (h); V = Container volume (l); C' = Cell concentration in the control aquaria (cells l^{-1}).

Counting was done under a phase-contrast microscope.

In order to evaluate biodeposition production (feces and pseudofeces), we placed one mussel for 5 h in a 500-ml aquarium containing one of four different algae concentrations in a mesotrophic to eutrophic range (5 to 80 $\mu g\ l^{-1}$), with two replicates per treatment. All mussels were starved for 24 h prior to the beginning of the experiment. We used a mesotrophic range in order to measure pseudofeces production with a reasonable error margin which would have been impossible at lower Chl *a* and dry matter content concentrations. Before starting the experiment, the relationship between dry matter and Chl *a* content was determined by taking 100 ml from each aquarium and filtering it through a Millipore filter which had been dried at 60°C for 24 h. Samples were dried and weighted again after 24 h. We used a fine hose (4 mm diameter) and a small vacuum to carefully collect all particulate deposits from around the mussel and on the bottom of each aquarium, including control aquaria. This material was collected in clean bottles, filtered, dried and weighted as described above.

In order to evaluate the ability of mussels to recycle ammonium (NH_4), a short experiment was performed by placing six adult individuals in ten 500 ml aquaria filled with filtered lake water. All the mussels had been feed for 8 h before the experiment. We took one 100 ml sample from each aquaria at the beginning of the experiment to measure NH_4 concentration; after 1 h we took another 100 ml sample from three aquaria. The remaining seven aquaria were left for 6 h, when we took samples from three of them and left the remaining four for three more hours. This experiment was performed in the dark to avoid phytoplankton growth.

2.3. Field mussel sampling

The mussel sampling was performed at Totoral bay under and among salmon cages (13 to 18 m depth). In order to collect the mussels, two divers took eight random sediment samples with a cubic frame (50 cm^3) which was inserted in the substrate. All the mussels were collected from each sample and measured. The samples were taken under three experimental salmon pens which produced a sediment shadow of approximately 5 m^2. These pens were at least 400 m apart. Two control sites of same area, separated by approximately the same distance, were selected and similarly sampled on five occasions, between January and December 1995.

3. Results

3.1. Outdoor tank experiments

Minimum daily water temperatures ranged from 11.2 to 12.3°C, while maximum temperature during the whole period ranged from 15.3 to 16.9°C with highest values in January. There was no difference between treatments regarding temperature and, overall, tanks were exposed to daily temperature variations of 4 to 5°C. pH values were slightly higher in the salmon culture tanks (8.1) while in the other tanks the average was 7.6.

Average pH per treatment were obtained by averaging H^+ concentration and then converting the values back to pH. However, no statistical differences were detected among treatments with either the two-way ANOVA or the pairwise tests (*t*-test or Tukey). Likewise, while oxygen values in each tank decreased slightly toward the bottom, slow stirring conducted twice a day seemed to be sufficient to avoid oxygen differences among tanks and oxygen depletion related to treatments. Oxygen values ranged from 7.1 to 8 mg l^{-1} when measured early in the morning.

The ANOVA table and statistical significance of each factor effect on water quality parameter are shown in Table 2, where it can be observed that there was a very strong effect of mussels on all parameters as well as a salmon effect.

The initial total phosphorus (TP) concentrations in all the tanks were fairly high, most likely because the spring water is groundwater and rich in P. This can also explain the very low initial Chl*a* concentrations. Eighteen days after the start of the experiment the presence of mussels had a significant effect on most variables measured (Table 2). However, the most dramatic effect was for TP, which increased from ~ 30 μg l^{-1} to more than 200 μg l^{-1} in the water column of tanks with salmon (Table 2). This value was significantly greater than TP levels in the salmon–mussel treatment ($P < 0.001$). However the TP concentration also increased in the control tanks, reaching about half the concentration in the salmon tanks (Table 2).

During the experiment we added 480 mg of TP daily through fish food to each tank. Approximately 90 mg of this remained in the water column in tanks without mussels during the whole experiment (Table 2). This accounts for 200 to 230 μg l^{-1} of TP in the water column based on the tank water volume of 450 l (Table 2). In tanks with mussels, 18 days after the beginning of the experiment, none of the added TP remained in the water column as the concentration was less than at the beginning of the experiment and also lower than in the control tanks. However, after 61 days, 38 mg of TP was observed in the water column.

Similar effects of salmon and mussels were observed for Chl*a* concentration in the tanks. Eighteen days after the experiments began, Chl*a* reached the hypereutrophic average levels of 336 μg l^{-1} in the salmon tanks while the salmon–mussel tanks averaged 23 μg l^{-1} (Table 2). According to these data, it is estimated that each of the 35 mussels filtered about 0.5 μg of Chl*a* or more daily.

Values of NH_4 were also significantly reduced by mussels, especially 18 days after the beginning of experiment. NH_4 concentrations later increased in the salmon mussel treatment and differences among this treatment and the salmon alone treatment became non-significant (Table 2).

At day 102, two of the three salmon–mussel tanks in which *Diplodon* was removed, showed more than a 2-fold increases in Chl*a* (Fig. 1), while the three mussel tanks where *Diplodon* was removed showed between 4- and 19-fold Chl*a* increases. Two of the three salmon tanks where *Diplodon* was added showed a steep decrease in Chl*a* concentration to values below 3 μg l^{-1} (Fig. 1). Most *Diplodon* in the third tank died soon after introducing them, possibly due to bad handling. Chl*a* concentrations did not show any particular trend in the control tanks.

Concentrations of nutrients in the sediments did not show significant treatment effects (Table 3). One exception occurred on day 39 when there was a marginal mussel effect

Table 2
Tank experiments with mussels (*D. chilensis*) and salmon (*O. kisutch*)

Parameter	Treatments	Initial	18 days later	39 days later	61 days later
P total ($\mu g\ l^{-1}$)	**control**	34.5 ± 6.7 ns	62.5 ± 4.1 *	140.3 ± 24.4 ns	127.6 ± 15.6 +
	mussel	37 ± 4.2	7.3 ± 1.2	21 ± 1.5	19.2 ± 2.6
	salmon	35.3 ± 2.1 ns	205.3 ± 31.7 * * *	345 ± 72.7 * *	237.2 ± 19.5 +
	salmon–mussel	39.9 ± 4.3	19.8 ± 2.2	99.8 ± 21.8	123.3 ± 48.9
ANOVA	mussel		0.001	0.002	0.005
P values	salmon		0.001	0.007	0.004
	interaction		0.004	0.153	0.925
PO_4 ($\mu g\ l^{-1}$)	**control**	24 ± 1.3 ns	10.7 ± 2.7 ns	24 ± 4.9 *	27.6 ± 5.2 ns
	mussel	18.5 ± 1.2	1.7 ± 0.8	0.5 ± 0.5	1.0 ± 0.7
	salmon	16.3 ± 8.7 ns	63.1 ± 7.4 * * *	37.8 ± 4.6 ns	35.6 ± 14.7 ns
	salmon–mussel	22 ± 4.4	3.9 ± 0.9	19.6 ± 7.7	24.5 ± 17.8
ANOVA	mussel		0.001	0.004	0.221
P values	salmon		0.001	0.012	0.150
	interaction		0.001	0.618	0.532
NO_3 ($\mu g\ l^{-1}$)	**control**	243 ± 46.2 ns	81.2 ± 7.5 ns	167.8 ± 7.5 ns	18.7 ± 9.4 ns
	mussel	212 ± 111	69.7 ± 15.5	169.8 ± 9.9	0.8 ± 0.5
	salmon	257 ± 33 ns	90.5 ± 9.9 *	172.3 ± 7.8 ns	43.5 ± 1.6 ns
	salmon–mussel	224 ± 41	19.3 ± 9.3	154.5 ± 1.3	21.7 ± 15.8
ANOVA	mussel		0.006	0.261	0.06
P values	salmon		0.1	0.435	0.036
	interaction		0.026	0.170	0.874
NH_4 ($\mu g\ l^{-1}$)	**control**	0.5 ± 0.5 ns	3.93 ± 2.2 ns	20.7 ± 6.9 ns	22.7 ± 6.1 ns
	mussel	0.5 ± 0.5	0.73 ± 0.7	11 ± 7.7	4.3 ± 2.5
	salmon	0.5 ± 0.6 ns	44.4 ± 3.7 * * *	89.1 ± 22.1 * *	40.2 ± 15.4 ns
	salmon–mussel	1.2 ± 0.6	2.8 ± 1.5	16.8 ± 5.4	24.5 ± 10.3
ANOVA	mussel		0.001	0.011	0.124
P values	salmon		0.001	0.018	0.093
	interaction		0.01	0.037	0.89
Chlorophyll *a* ($\mu g\ l^{-1}$)	**control**	1.8 ± 0.6 ns	110.0 ± 9.7 * *	47.5 ± 23.7 ns	51.1 ± 9 * *
	mussel	1.5 ± 1.1	2.7 ± 0.3	2.1 ± 0.1	2.1 ± 0.4
	salmon	1.5 ± 0.8 ns	336.0 ± 44.9 * * *	312.3 ± 109 +	124.3 ± 16.3 * *
	salmon–mussel	1.9 ± 0.6	23.0 ± 4.9	87.5 ± 26.5	42.7 ± 10.2
ANOVA	mussel		0.001	0.046	< 0.001
P values	salmon		0.001	0.016	0.001
	interaction		0.002	0.156	0.163

Average values of 3 replicates ± 1 s.e., for some water quality parameters in the different treatments, before and on three dates after the beginning of the experiment. The statistical significance of the two-way ANOVA for the 'salmon' and 'mussel' treatments, with two levels for each factor and three replicates per treatment is indicated for each parameter. Also statistical significance of pair wise comparisons: control vs. mussel, and salmon vs. salmon–mussel is indicated. $P \geq 0.1$ (ns), $0.05 < P < 0.1$ (+), $P \leq 0.05$(*), $P \leq 0.01$(* *), $P \leq 0.001$(* * *).

on total nitrogen (TN) concentrations ($P = 0.058$). In general, total phosphorous (TP) concentration increased in all experimental tanks due to allochtonous inputs from the surrounding vegetation and wind driven particles, which seemed to override the fish feed input. On the contrary TN concentrations decreased in the sediments through time,

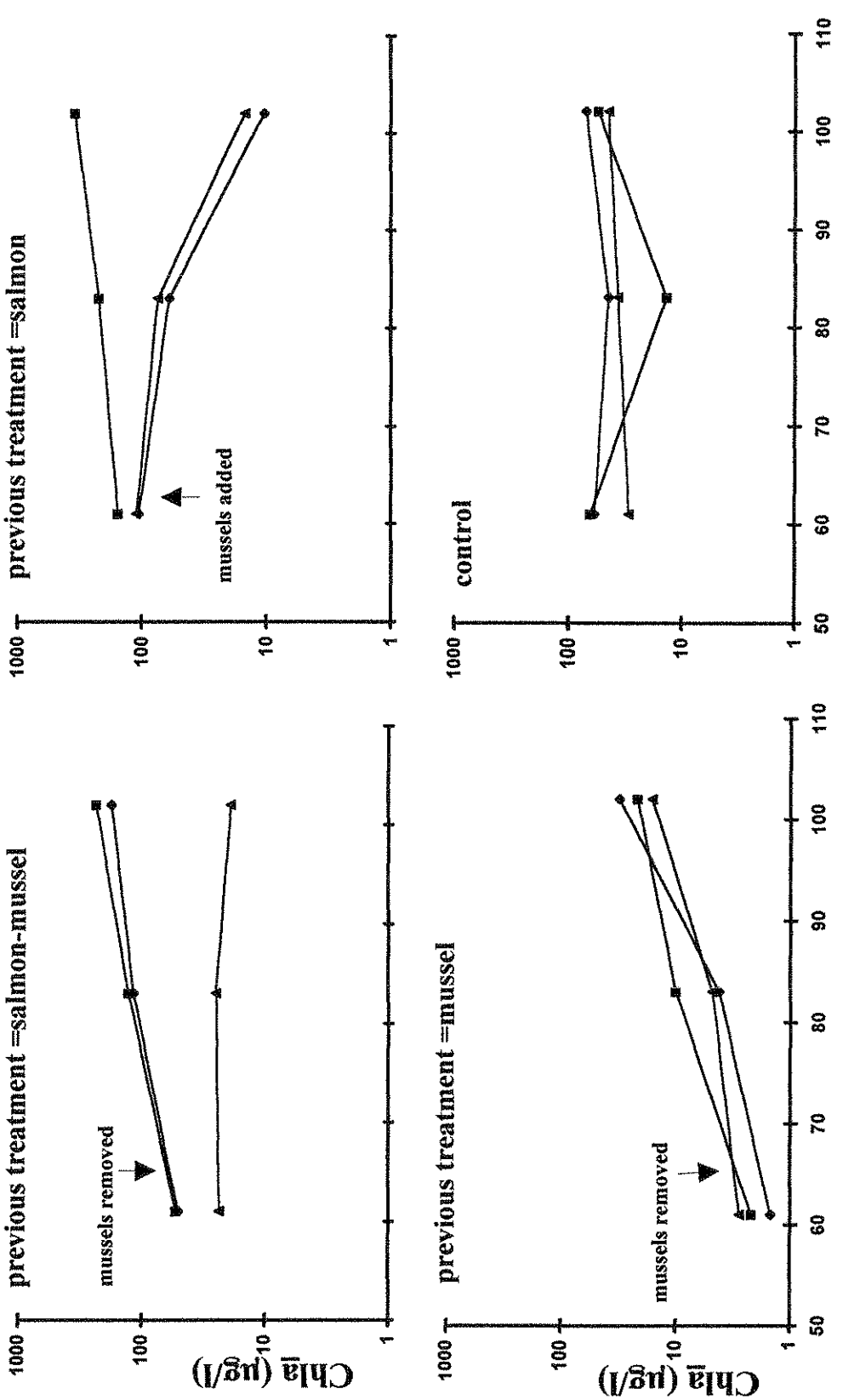

Fig. 1. Tank experiments changes in chlorophyll *a* concentration through time after removal and addition of mussels. Control tanks remained undisturbed. Each line represents a single tank.

Table 3
Average concentration (ppm) ± 1 s.e. of sediment total phosphorous (TP) and total nitrogen (TN) in the different treatments

Treatments					ANOVA statistical significance		
Salmon–mussel		Salmon	Control	Mussel	Salmon	Mussel	Interaction
18 days	TP 88 ± 7.7	87 ± 13	95 ± 4	69 ± 29	ns	ns	ns
	TN 503 ± 263	687 ± 141	630 ± 326	659 ± 274	ns	ns	ns
39 days	TP 355 ± 11.5	333 ± 35	345 ± 8	222 ± 106	ns	ns	ns
	TN 840 ± 153	335 ± 84	748 ± 173	255 ± 6.3	ns	+	ns
61 days	TP 442 ± 130	455 ± 95	565 ± 61	362 ± 114	ns	ns	ns
	TN 440 ± 88	357 ± 86	528 ± 18	231 ± 39	ns	ns	ns

Statistical significance of the ANOVA for the two factors (as shown in Table 1) is indicated, $P \geq 0.1$ (ns), $0.05 < P < 0.1$ (+). Initial TP = 77 ± 12 ppm, Initial TN = 1267 ± 34 ppm.

possibly revealing bacterial activity which was not evaluated during this experiment (Table 3).

Diplodon condition index varied between 0.198 and 0.324 at the beginning of the experiment. At that time, no significant differences were present among the condition index in salmon tanks and those in mussel alone tanks. At the end of the experiment, a two way ANOVA considering salmon and size classes as the two factors showed no effect of salmon on the condition index, but there was a size class effect (Table 4). A similar analysis was performed on the differences between condition index after and before the experiment with similar results: no effect of salmon ($P = 0.104$), a significant effect of size class ($P = 0.004$) and no interaction effect ($P = 0.837$). In general the largest individuals in all tanks lost some weight, while the smallest individuals gained weight.

3.2. Aquarium experiments

There was a positive significant correlation between algal cell concentration and Chl *a* concentration ($r^2 = 0.83$, $P = 0.022$), and adult *D. chilensis* reached maximum cell

Table 4
Average condition index (K) ± s.e., for *D. chilensis* at the end of the experiment for each size class in the salmon–mussel tanks and in the mussel alone tanks

Mussel	Treatment		Two-way ANOVA	
Size class (cm)	Salmon–mussel	Mussel	Factor	P
< 4	0.218 ± 0.004	0.217 ± 0.007	salmon	ns
4–4.99	0.218 ± 0.004	0.214 ± 0.003	size class	***
5–5.99	0.203 ± 0.002	0.197 ± 0.004	interaction	ns
> 6	0.194 ± 0.004	0.198 ± 0.004		

Significance of the two-way ANOVA (salmon and mussel size effects, $P \geq 0.1$ (ns), $P \geq 0.001$ (***).

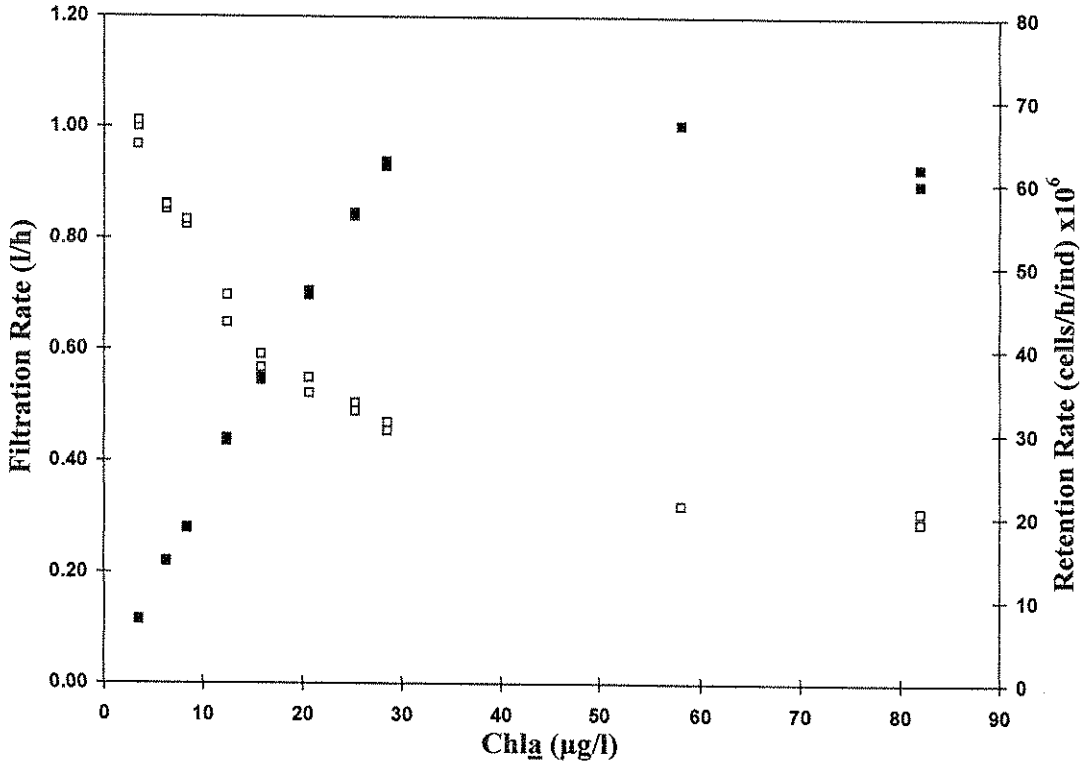

Fig. 2. *D. chilensis* filtration rate (l h^{-1}) (open symbols) and retention rate (cells h^{-1}) (filled symbols) over a wide chlorophyll *a* (Chl*a*) concentration range.

retention of 60×10^6 cells ind^{-1} h^{-1} at Chl*a* concentrations between 20 and 30 µg l^{-1} (Fig. 2). An adult retains about 9×10^6 cells h^{-1} when plankton concentration is in the order of 10 million cells per liter (4 to 5 µg l^{-1} Chl*a*; Fig. 2). At Chl*a* values less than 30 µg l^{-1}, retention rate can be described as a linear function according to:

$$\text{Retention Rate}\left[\text{cells}\,h^{-1}\,\text{ind}^{-1}\right] = 0.911 \times \text{algal concentration}$$

Under similar conditions, filtration rate behaves as a negative exponential function of Chl*a* concentration (or cell density): Filtration rate [1 h^{-1} ind^{-1}] = 5.15 − 0.59 × ln [cells l^{-1}]. That is, at average maximum Chl*a* concentration in Lake Llanquihue (2 µg l^{-1}), an adult *D. chilensis* filters about 1 l h^{-1} if other conditions are appropriate (Fig. 2).

Under the experimental conditions, there was a strong correlation ($r^2 = 0.89$; $n = 8$) between total seston in the water and daily biodeposit production. This relationship was:

$$\text{Biodeposit Production}\left(\text{mg}\,\text{ind}^{-1}\,\text{day}^{-1}\right) = 1.077 + 0.376 \times \text{Total Seston}\left(\text{mg}\,l^{-1}\right)$$

Because total seston and Chl*a* were highly correlated ($r^2 = 0.97$, $n = 8$), biodeposit production could also be expressed in terms of Chl*a* concentration as:

$$\text{Biodeposit Production}\left(\text{mg}\,\text{ind}^{-1}\,\text{day}^{-1}\right) = 1.312 + 0.023 \times [\text{Chl}a].$$

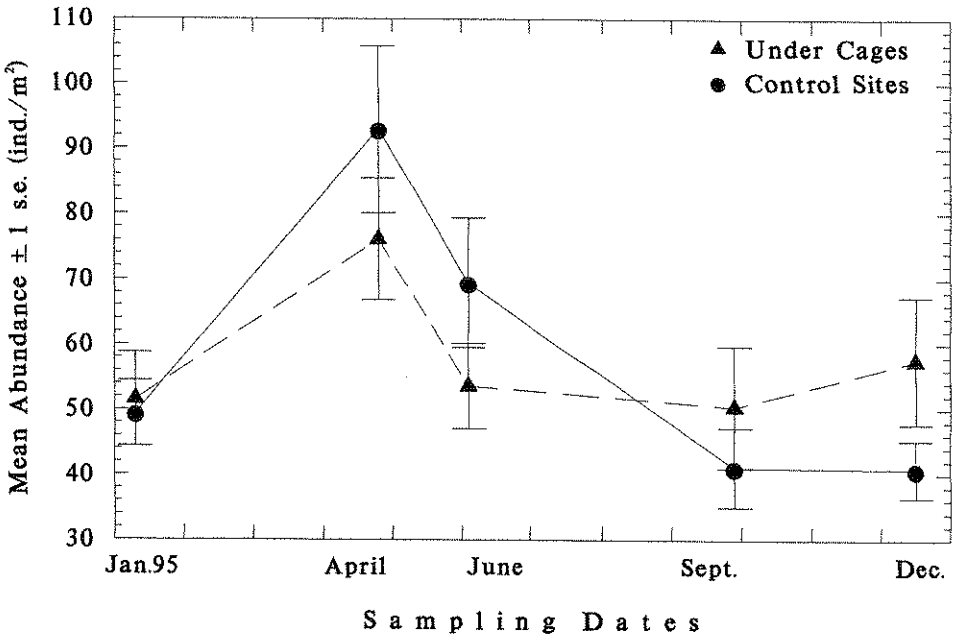

Fig. 3. Average densities ±1 s.e. of *D. chilensis* under three salmon cages and at two control sites, Lake Llanquihue, Chile.

Adult *Diplodon* produced 7.9 ± 4.8 (s.e.) μg of NH_4 during the first hour after a day of feeding in an environment with 10 to 12 μg of Chl*a*.

3.3. Field measurements

The first mussel sampling in January 1995 showed very similar densities (around 50 ind m^{-2}) under salmon pens and in control sites. Subsequent mussel densities fluctuated through time with similar trends in both salmon pens and control sites (Fig. 3). In April, control densities reached an average of 93 ind m^{-2}, while under salmon cages these densities were lower, yet these differences were not statistically significant ($P > 0.10$, *t*-test). Later, densities declined at all sites and no significant differences were ever found among mussel densities under salmon cages and in control sites.

4. Discussion

4.1. Effects of mussels on water quality and sediments

In the tank experiments *D. chilensis* changed a hypereutrophic situation resulting from salmon culture to an oligotrophic–mesotrophic one. However, because the tanks were closed systems, the effect of mussels declined by day 61, probably due to excessive accumulation of organic matter. Mussels were even able to reduce Chl*a* and

other nutrients to much lower values than in control tanks (Table 2), where an eutrophic situation originated from the high nutrient concentration in the spring water used to fill all the tanks.

Laboratory experiments corroborated results from tank experiments, *D. chilensis* cleared the water through a combination of direct consumption of particulates and by biodeposition.

Considering mussels, relatively high filtering rate at low Chl*a* concentrations (Fig. 2), such as those in the field (Table 1), and their high density in Chilean lakes, particularly in coastal areas and bays (50 to 200 ind m^{-2}), they may exert a major effect on some lakes. We calculated that given the present densities and size distribution of these mussels, they could filter the water of Totoral Bay (3.98×10^6 m^2 of area and 9.95×10^{10} l in volume) in about 2 weeks. That is, if they were continuously filtering and if the water column was circulating across the bottom to give them access. The latter is a fair assumption since the epilimnion, or warmer water column, is very deep in this lake (greater than 30 m). Most of the water column in the bay does not stratify in summer, which means there is circulation down to the bottom (Mena, 1997).

In clearing the water column, the mussels are capturing particulate material at the bottom sediments.

Assuming Chl*a* average values of 3 $\mu g\ l^{-1}$, these bivalves in the 3.98 km^2 Bay could produce a daily total of 329 kg of biodeposits which means a net particle flux to the sediments of 175.8 tonnes per year.

Considering that the salmon farming activity in this bay is about 200 tonnes of salmon a year with a conversion efficiency of 1.2, approximately 150 tonnes of allochtonous sediments are produced annually, based on approximately 25% of the fish biomass is dry weight (Enell, 1987; Beveridge, 1996). Additionally, about 3.2 tonnes of P are left in the environment, approximately 7 kg daily, if culture conditions are constant through the year. About 60% of this P falls directly to the bottom and 40% is left in the water column (Enell, 1987). Clearly, with the filtering ability and densities of *D. chilensis* in the bay, a large portion of the P should quickly set to the bottom as well as a large proportion of the N, especially particulate organic forms.

During the tank experiment there should have been a net P loss from the water column to sediments particularly in the salmon–mussel tanks, due to the ability of mussels to remove particles from the water column. However, this effect was not clear when analyzing sediment TP content since concentrations did not show treatment effects (Table 3). Possibly, the much greater P inputs from the surrounding vegetation had a confounding effect which does not allow us to discern the response of sediments to the nutrient inputs due to experimental manipulations.

One concern with the increase in the deposition of particulates including TP to the bottom is the potential accumulation of organic matter under salmon cages, this may create unwanted effects such as anoxic conditions, production of toxic gases etc. (Beveridge, 1996). However, mussels help to prevent the accumulation that occurs just under the cages, since they move through the bottom causing bioturbation of the water–sediment layer (Lara and Moreno, 1995; Mena, 1997). At every sampling, the divers observed many trails produced by moving mussels under and beyond cages. Perhaps for the same reason, the distribution of *D. chilensis* was not affected by salmon

cages in the experimental site at Lake Llanquihue (Fig. 3), suggesting that their effect is evenly distributed.

Some bivalves are efficient at nutrient cycling and mineralization by uptaking particulate organic nitrogen and releasing ammonium (Yamamuro and Koike, 1993). These authors showed that *Corbicula japonica* increased fluxes of dissolved inorganic forms of N and P from the sediments to the water column. Indeed, ammonium excretion is one way bivalves enhance primary productivity even though at the same time they are removing primary producers, and thus they may be able to maintain a high rate of primary productivity (Yamamuro and Koike, 1993).

In preliminary aquaria experiments, *Diplodon* produced about 8 μg of NH_4 per hour. Therefore, it can be expected that *Diplodon* will enhance primary production, but without allowing accumulation of phytoplankton biomass in the water column due to its filtration capacity.

In the natural lake environment, the role of mussels in changing the N:P ratio in the water column could also be relevant. This could occur by reducing P concentration more than N concentration and also by exporting ammonia back to the water column. In this context, *D. chilensis* could play a key role in maintaining oligotrophy, both by changing nutrient ratios and also by controlling phytoplankton densities. It is important to mention that the filtering ability of *Diplodon* at the low Chl*a* range found in the lake could prevent phytoplankton growing above oligotrophic levels. Both mechanisms should be explored more deeply in the field to understand their relevance.

4.2. Biomanipulation to mitigate salmon farming effects

Attempts to culture bivalves together with pen-salmon farming have been made, particularly in marine environments where the availability of commercial bivalve species offers possibilities for polyculture (Stirling and Okumus, 1995).

In freshwater environments, however, biological possibilities for mitigation of the environmental impacts of pen-salmon farming are less well known. There are fewer bivalve filter feeders, and almost none with commercial value, unlike *Diplodon*.

D. polymorpha is well known for its high filtering capability and density (Hebert et al., 1991), but *D. chilensis* has comparable filtering ability. This is mainly due to its greater size rather than the abundance it achieves in a particular bay, although biomasses (20–100 g m^{-2}) are comparable to those of the much smaller but more numerous *Dreissena* (MacIsaac, 1996).

These mussels contribute to increased bottom heterogeneity because they are large and long lived, and because they stand upright on the bottom, protruding 2 or 3 cm above the sediments. These mussel beds may contribute to increasing macrocrustacean abundance and attract fish (Fig. 4). A native freshwater crab *Aegla* sp. feeds on the mussels (Lara and Moreno, 1995), also a common freshwater shrimp, *Samastacus spinifrons*, feeds actively on them as soon as they open the shell. Native fish and free-living salmonids are attracted to these areas and feed on macrocrustaceans *Aegla* and *Samastacus* and on aquatic insects (Palma, 1996). Thus, the mussels provide energy and a nutrient source to the benthic and pelagic foodweb (Fig. 4) contributing to a more rapid recycling of organic matter and nutrients. In general mussels are more dense in

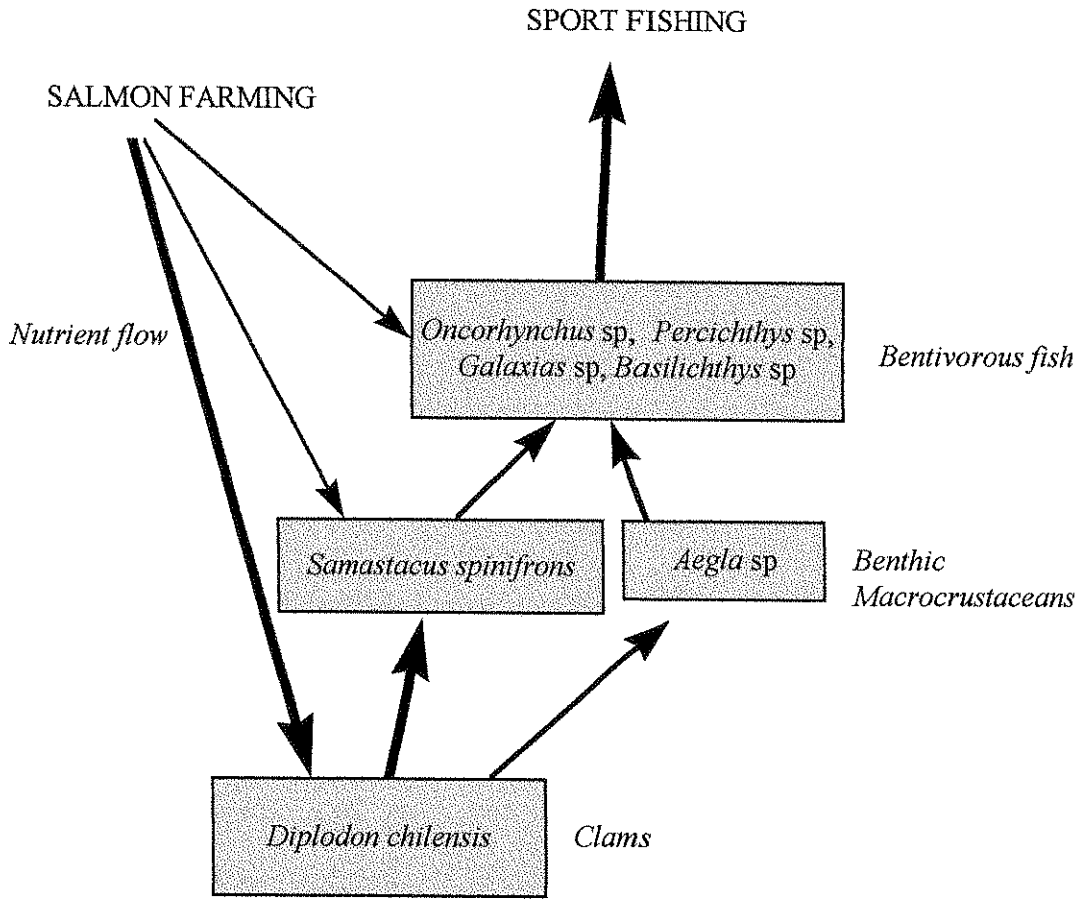

Fig. 4. Nutrient flow and trophic interactions linking the mussel *D. chilensis* and salmon farming in southern Chilean lakes.

areas with greater organic matter input, for example in bays with salmon farming or in areas with sewage outflows, although they do not concentrate around the source points but spread around (Lara and Parada, 1988). Finally, enhanced fish biomass is observed in these bays, which in turn, increases sport fishing (Palma, 1996), crayfish production and potential harvesting (Fig. 4). Our estimates of fish biomass in the bays with salmon farming are on average 30 to 40% greater than those of bays without salmon farming, and approximately 70% of the fish food is taken from the sediments (Soto, pers. observation).

It is possible to encourage the location of salmon cages in sandy and fine gravel sediments which are appropriate for *Diplodon* settling. It may even be possible to increase *Diplodon* populations or to create new populations under salmon cages.

D. chilensis may also be considered for use as biological cleaning unit in sewage sludge outflows, where they can contribute to settling of particulates and bacteria. They are able to withstand heavy nutrient loading conditions (Table 4) and are able to move freely in the sediments (Lara and Parada, 1988) avoiding oxygen depletion. Thus, *Diplodon* can remain in large pans or cages at the bottom of settling tanks and be replaced every 4 to 6 months to ensure maximum filtering effects.

We conclude that it is possible to improve the environmental situation of salmon farming in freshwater lakes from a better knowledge of the benthic environment and by finding ways to couple benthic and pelagic communities such as the case shown here.

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