CHAPTER 24

The Patagonian blenny (*Eleginops maclovinus*): a Chilean native fish with potential to control sea lice (*Caligus rogercresseyi*) infestations in salmonids

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24.1 Introduction

The salmon farming industry in Chile started in 1978 and evolved over three decades to become the fastest growing industry in Chile, and the second largest producer after Norway from 1992 (Bjørndal, 2002; Niklitschek *et al.*, 2013). The main salmon production in Chile is located in the X region, around the city of Puerto Montt and Chiloé Island. However, this area extends over regions XI and XII. Due to the physical characteristics of the coast, these regions offer protected sites with ideal temperature and salinity levels. Additionally, a large number of rivers and lakes offer ideal conditions for production of salmon smolts all year around (Bjørndal, 2002). The main species farmed in Chile is Atlantic salmon (*Salmo salar*). However, rainbow trout (*Oncorhynchus mykiss*) and Pacific salmon (*Oncorhynchus kisutch*) are also produced. The total salmonid production in 2006 reached a maximum of 645,000 tonnes, with an economic return of US$2.2 billion (IFOP, 2015; SERNAPESCA, 2015). This production level was reduced in 2007 in response to the health crisis produced by the ISA virus (Infectious Salmon Anaemia). After implementation of adequate measurements, production levels recovered. In 2014, 955,000 tonnes of salmonids were harvested (SERNAPESCA, 2015).

Sea lice are copepod ectoparasites with a worldwide distribution that affect wild and farmed marine fish species (Farlora *et al.*, 2016). *Caligus rogercresseyi* is the dominant sea louse found in the salmon and trout farms in Chile (Fig. 24.1). The presence of this parasite was first described during the early stages of the Chilean salmon farming industry. *C. rogercresseyi* was transmitted by native fish species such as *Eleginops maclovinus* and *Odonthestes regia*, common wild hosts to this parasite (Gonzalez and Carvajal, 2003). Gonzalez and Carvajal (2003) determined that *C. rogercresseyi* has eight development stages, including three planktonic stages, two nauplius and a copepodid (infective stage) that settle on the host utilising a pair of antennae. Once attached to the host, the parasitic stages of *C. rogercresseyi* comprise four different chalimus stages and then adult females and males (Gonzalez and Carvajal, 2003). Although sea lice infestations do

Fig. 24.1 Female *Caligus rogercresseyi* with egg strings attached to Atlantic salmon. Credit: The Copepodite Production Laboratory, Quillaipe Research Station, Fundación Chile.

not directly cause mortality, this parasite causes skin damage and induces stress response in salmonids, which generates immune depletion that makes fish more prone to further infections (Valenzuela-Miranda *el al.*, 2015). The cost of medicinal treatment alone utilised to control sea lice by the Chilean salmon industry was almost US$80 million in 2013, while the total economic disturbance related to sea lice (that is operation costs, reduction of fish quality, among others) was estimated to be about US$300 million in the same year (Agusti *et al.*, 2016) .

The Chilean National Service of Fisheries and Aquaculture (Sernapesca) classified Caligus infestations in 2007 as a High Risk Disease, due to the severity of infection, and therefore it can be included in health programmes of surveillance and control. A new vigilance and control strategy for Caligus infestations was implemented in 2012. This strategy is regulated through the Caligidosis Specific Sanitary Programme, as indicated in the regulatory resolution N° 1141. This strategy is based on the principles that Sernapesca has established for all aquaculture disease control programmes, such as early detection of cases, timely control of infested animals, and protection of healthy fish.

Currently there is no specific regulation for the use of cleaner fish in Chile, and there are no private companies using this technology. Sea louse infestations in Chile have been historically controlled with medicines applied either by bath or by oral treatments. These medicines include organophosphates, Avermectins (ivermectins and emamectin benzoate), pyrethroids (deltamethin and cypermethrin), chitin inhibitor (diflubenzuron). However, sea lice have developed resistance to most of these anti-louse medicines used in Chile (Agusti *et al.*, 2016). However, Sernapesca is encouraging the use of non-pharmacological treatments as part of the Caligus treatment strategy. Salmon farming companies, on the other hand, focus on sustainability by a reduction in use of medicines and to find a cost-effective equilibrium of non-pharmacological treatments.

24.2 The Patagonian blenny

The Patagonian blenny (*Eleginops maclovinus*) is a protandrous hermaphrodite, notothenoid fish belonging to the family Eleginopidae (Lincadeo *et al.*, 2006) (Fig. 24.2). *E. maclovinus* is distributed from Valparaiso (33° 00' S) to the Beagle Channel (54° 00' S) in the Pacific Ocean and from southern Argentina and the Falkland Islands to the coasts of Uruguay in the Atlantic Ocean (Henriquez *et al.*, 2011). This fish inhabits coastal areas, river mouths and estuaries, and exhibits characteristics of a euryhaline and eurythermic species. The Patagonian blenny during its life cycle will first sexually mature as a male and later undergo a sexual reversion process to become female individuals. This phenomenon has been described in populations from different locations. Studies have estimated size and age of sexual reversion and this varies between 35 and 53 cm length, and two and six years of age, depending on geographic locations (Ceballos, 2011). Males and females become sexually mature in August, and the main spawning season occurs during September and December in sandy estuarine areas (Brickle *et al.*, 2005). A male generally generates 9 mm^3 of seminal fluid. Spermatozoa become active when mixed with secretion from spermatic conducts. Average flagellar motility is for 1.5 hours at 17°C. Milt show a circular-shaped head (1.9 µm diameter) with an intermediate segment (1.1 µm length) and a tail of 27.4 µm average length. Patagonian blenny eggs are pelagic, spherical shaped (1.9 mm diameter), transparent and with one oily droplet.

Juvenile and adult Patagonian blenny inhabit estuarine areas and generally prey on benthic invertebrates, such as polychaetes and small crustaceans and insects. However, results from studies describing the stomach content of captured specimens have reported the presence of significant amounts of different types of vegetables. Authors agree, however, that Patagonian blenny selectively, or opportunistically, prey mainly on animal items (Sa *et al.*, 2014).

Patagonian blenny potential as cleaner fish

Trials aimed at assessing the growth potential of Patagonian blenny under sea cage conditions suggested that this species was able to graze on sea lice attached to the skin of counterparts sharing the same cage (P. Saez, Estación Experimental Quillaipe, Fundación Chile, February 2017, personal communication). Based on this finding, Fundación Chile has developed and conducted two projects aimed at assessing the sea lice grazing potential of Patagonian blenny when cohabitated with Atlantic salmon in tank conditions. Another objective was to determine the effect of

Fig. 24.2 Patagonian blenny used in cohabitation trials at the Quillaipe Research Station. Credit: Fundación Chile, Puerto Montt.

culture density and the blenny: salmon ratio on sea lice cleaning efficiency in both tanks and sea cages. The ability of Patagonian blenny to ingest salmon feed of different sizes was also assessed. The aim of this chapter is to describe the results of these trials by researchers at Fundación Chile aimed at assessing the potential of Patagonian blenny as cleaner fish against sea lice infestation.

24.3 Trials assessing the potential of Patagonian blenny as cleaner fish

24.3.1 Tank-based trials

All tank-based trials were conducted at the Estación Experimental Quillaipe, Fundación Chile, Puerto Montt, Chile. Trials were carried out by the R&D unit of the technical department of Marine Harvest Chile, and Aquadvise, during the conduct of project 12IDL1-13173 INNOVA-CORFO 'Biological treatment against sea lice (*Caligus sp.*) in farmed salmonids' and :INNOVA-CORFO project L2 'Development of a biological treatment against sea lice (*Caligus sp.*) in farmed salmonids'.

Patagonian blenny used in this trial were of wild origin, captured from the Hueihue Bay, Chiloé, X Región. Fish were kept following capture under culture conditions for a period of 12 weeks prior to commencement of the trials. During this period fish were treated with cypermethrin 1% (Betamax®) to ensure elimination of potential internal parasites.

C. rogercresseyi infestation

Fish were exposed to infestation with copepodites at the beginning of the trial. Fish were kept off feed for a period of 24 hours prior to the beginning of the infestation. Copepodites used in the trial were generated from adult females with egg strings of *C. rogercresseyi* isolated from Atlantic salmon kept at the Quillaipe Research Station. Adult females were transported to the Copepodite production laboratory and incubated in sea water (32‰, filtrated at 88 μm and UV treated). Water temperature was kept at 12–14°C with no light and constant aeration. Infestations were conducted when 90% of the individuals in the planktonic stage reached the copepodite stage. This procedure was conducted according to the protocol PO-AQ-16-LAB 'Procedures of production and infestation with C. rogercresseyi'. The experimental period for each trial was 35 days. Experimental tanks were kept in a saltwater flow-through system. Tanks were supplied with faeces collection systems provided with a mesh for parasite collection. The number of sea lice per fish was assessed at seven-day intervals.

The objective of Trial 1 was to evaluate the Patagonian blenny as a cleaner fish of Caligus infestation in Atlantic salmon. The hypothesis was: do Patagonian blenny show cleaner activity when kept in a cohabitation model with Atlantic salmon in tanks? The Patagonian blenny were of initial average body weight of 60 g (wild origin), and Atlantic salmon had an initial average body weight of 155 g**.** The treatments were:

- Treatment 1 (blenny:salmon ratio = 20%) = 25 Atlantic salmon + 5 P. blenny + *C. rogercresseyi*
- Treatment 2 = 30 blenny + *C. rogercresseyi*
- Treatment 3 (Control) = 30 Atlantic salmon + *C. rogercresseyi*

There was a 48.8% cleaning efficiency using Patagonian blenny (see Fig. 24.3 Treatment 1, for statistics). It was concluded that Patagonian blenny graze on *C. rogercresseyi* attached to Atlantic salmon when reared in a cohabitation model. The *C. rogercresseyi* infestation in Patagonian blenny was significantly lower than in Atlantic salmon. There were no significant differences in growth performance among Atlantic salmon reared in the cohabitation model

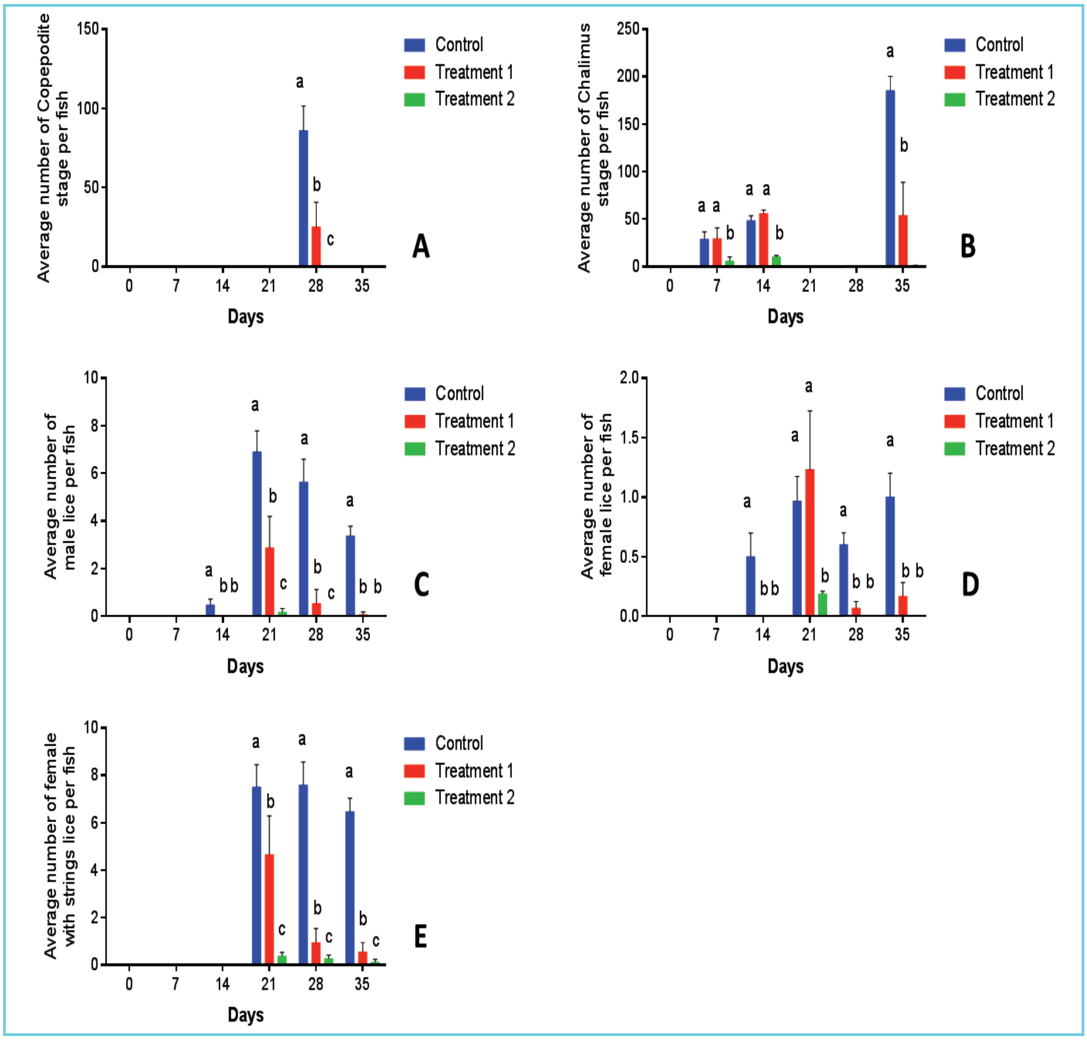

Fig. 24.3 Trial 1. Assessing sea lice cleaning activity of Patagonian blenny. Treatment 1 (P. blenny:salmon ratio = 20%) = 25 Atlantic salmon + 5 P. blenny + *C. rogercresseyi;* Treatment 2 = 30 P. blenny + *C. rogercresseyi;* Treatment 3 (Control) = 30 salmon + *C. rogercresseyi*. Average number of Copepodite (A), Chalimus (B), male (C), female (D), and female with strings (E) stages of *Caligus rogercresseyi* per fish recorded for each triplicate treatment during each sampling dates. Values are presented as means ± SD. Mean values which do not share a letter were found to be significantly different by Kruskal-Wallis and by Dunn's multiple comparisons test.

with Patagonian blenny or reared alone. Patagonian blenny grazed on *C. rogercresseyi* in the adult stage.

In Trial 2 the cleaning efficiency of Patagonian blenny on Caligus infestation in salmon reared at low culture densities was assessed. The hypothesis tested was that there are differences in the cleaning efficiency when different culture densities (based on initial and halfway culture densities observed in regular production cycles) were used. The fish used were blenny of average initial body weight = 82 g (wild origin) and Atlantic salmon were 138 g. The treatments were:

- Treatment 1 (P. blenny:salmon ratio = 10%, low culture density = 0.9 kg m^{-3}) = 50 Atlantic salmon + 5 blenny + *C. rogercresseyi*
- Treatment 2 = (P. blenny:salmon ratio = 10%, high culture density = 7.9 kg m^{-3}) = 460 Atlantic salmon + 46 blenny + *C. rogercresseyi*

The cleaning efficiency of blenny in Treatment 1 was 64.2%, compared with 93.6% in treatment 2 (Fig. 24.4). Therefore salmon stocking density had an effect on cleaning efficiency of Patagonian blenny, and on re-infestation of *C. rogercresseyi* in tanks. The greater level of sea lice reduction was observed at the higher stocking density of salmon. Not unexpectedly, salmon stocked at lower density had a higher growth performance.

In Trial 3 the cleaning efficiency of blenny in Caligus infestation was assessed at two stocking densities of blenny, 1:10 (10%) and 1:20 (20%) in tanks. The average weight of Patagonian blenny was 64 g (wild origin) and Atlantic salmon were 125 g. The treatment groups were:

- Treatment 1 = 500 Atlantic salmon only
- Treatment 2 = blenny:salmon ratio = 5% = 500 salmon + 25 blenny + *C. rogercresseyi*
- Treatment 3 = blenny:salmon ratio = 10% = 500 salmon + 50 blenny + *C. rogercresseyi*

The reduction in sea lice numbers was 60.4% in treatment 2 and 58.8% at the higher stocking ratio (Fig. 24.5). Therefore there were similar grazing levels in both treatment ratios.

The feed consumption of the blenny with different sizes of commercial salmon feed was examined in Trial 4. This work was funded by Innova Chile CORFO project L2 'Development of a biological treatment against sea lice (Caligus sp) in farmed salmonids'; with partners Marine Harvest Chile and Fundación Chile. The objective was to determine feeding rates of Patagonian blenny when fed Atlantic salmon commercial feed of different sizes during initial stages of culture cycle. The feed size 4, 6, 9, and 12 mm pellet were fed to blenny of three different body weight range. Each feed size was fed for a ten-day period. The treatments were:

- Treatment 1= blenny body weight range = 60–120 g
- Treatment 2 = blenny body weight range = 130–160 g
- Treatment 2 = blenny body weight range = 170–250 g

There was no feeding activity in any of the fish sizes when fish were fed with a pellet size of 9 and 12 mm. Fish fed pellet size 4 and 6 mm had higher feed consumption rates than those of fish fed pellet sizes 9 and 12 mm. Fish in the three weight ranges evaluated consumed the 4 and 6 mm pellet sizes.

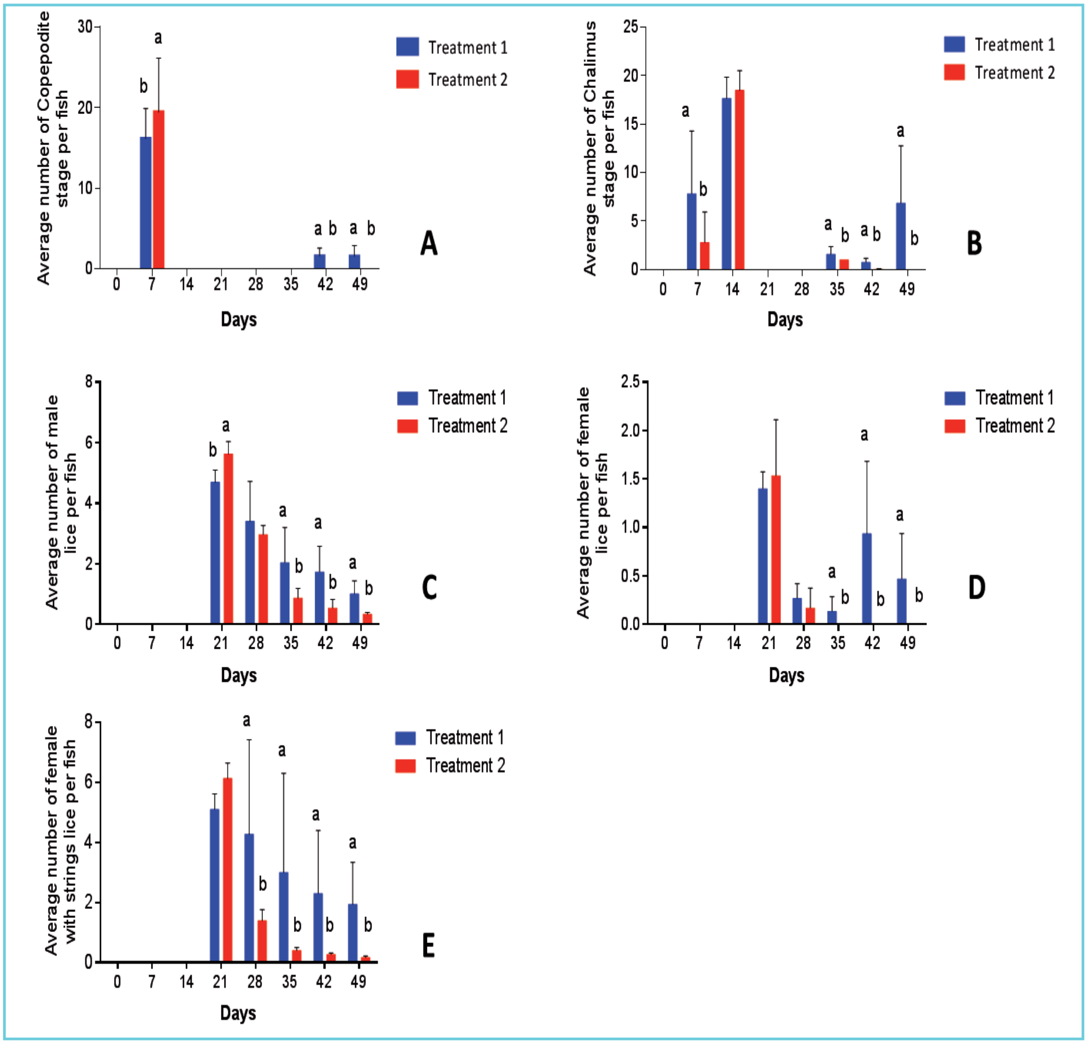

Fig. 24.4 Trial 2. Numbers of sea lice on salmon following stocking of blenny at two salmon densities. Treatment 1 (blenny:salmon ratio = 10%, low culture density = 0.9 kg m^{-3}) = 50 Atlantic salmon + 5 blenny + *C. rogercresseyi;* Treatment 2 = (blenny:salmon ratio = 10%, high culture density = 7.9 kg m^{-3}) = 460 Atlantic salmon + 46 blenny + *C. rogercresseyi.* Average number of Copepodite (A), Chalimus (B), male (C), female (D), and female with strings (E) stages of *Caligus rogercresseyi* per fish recorded for each triplicate treatment during each sampling dates in Trial 2. Values are presented as means ± SD. Mean values which do not share a letter were found to be significantly different by Kruskal-Wallis and by Dunn's multiple comparisons test.

24.3.2 Experimental trial in sea cage

All the sea cage trials (Trial 5) were conducted at the Huenquillahue Station, Marine Harvest Chile MHC, Puerto Montt by the technical department of MHC, the R&D of Quillaipe Research Station and Aquadvise, during the Innova Chile CORFO project L2 'Development of a biological

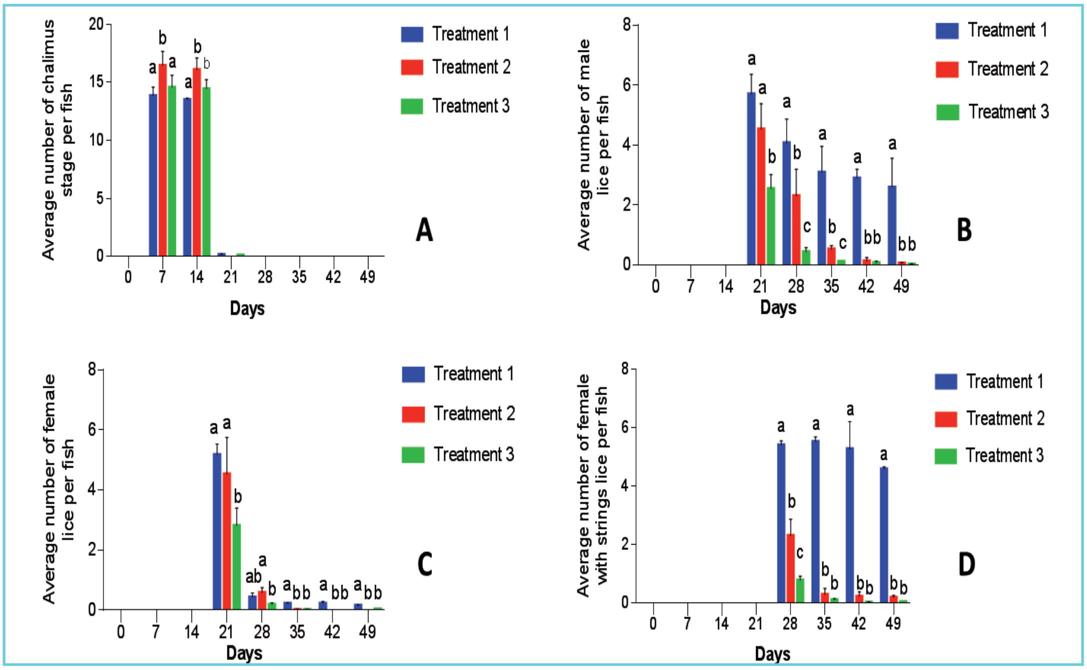

Fig. 24.5 The cleaning efficiency in Caligus infestation at two stocking densities of blenny, 1:10 (10%) and 1:20 (20%) in tanks. The average weight of Patagonian blenny was 64 g (wild origin) and Atlantic salmon were 125 g. Treatment 1 = 500 Atlantic salmon; Treatment 2 = blenny:salmon ratio = 5% = 500 salmon + 25 blenny + *C. rogercresseyi;* Treatment 3 = blenny:salmon ratio = 10% = 500 salmon + 50 blenny + *C. rogercresseyi.* Average number of Chalimus (A), male (B), female (C), and female with strings (D) stages of *Caligus rogercresseyi* per fish recorded for each duplicate treatment during each sampling dates in Trial 3. Values are presented as means ± SD. Mean values that do not share a letter were found to be significantly different by Kruskal-Wallis and by Dunn's multiple comparisons test.

treatment against sea lice (*Caligus sp.*) in farmed salmonids'. Patagonian blenny used in this trial were of wild origin captured from the Hueihue Bay, Chiloé, X Región. Fish were kept following capture under culture conditions for a period of 12 weeks prior to commencement of the trials. During this period fish were treated with brackish water (5 ppt) for 36 hours to eliminate sea lice. Fish were infected with naturally occurring sea lice at the Huenquillaue Station. The experimental period for each trial was 60 days and sea lice numbers were assessed at seven-day intervals.

In this Trial 5 the cleaning efficiency of blenny was assessed in salmon of different body weight under experimental conditions in sea cages. The treatments were:

- Treatment 1: blenny:salmon ratio = 10% = 150 Atlantic salmon (initial average body weight 500 g) + 30 blenny (initial body weight 70 g)
- Treatment 2: 150 Atlantic salmon (initial average body weight 500 g)
- Treatment 3: (blenny:salmon ratio = 20%) = 175 Atlantic salmon (initial body weight 3200 g) + 35 blenny (initial weight 70 g)
- Treatment 4: 175 Atlantic salmon (initial weight 3200 g).

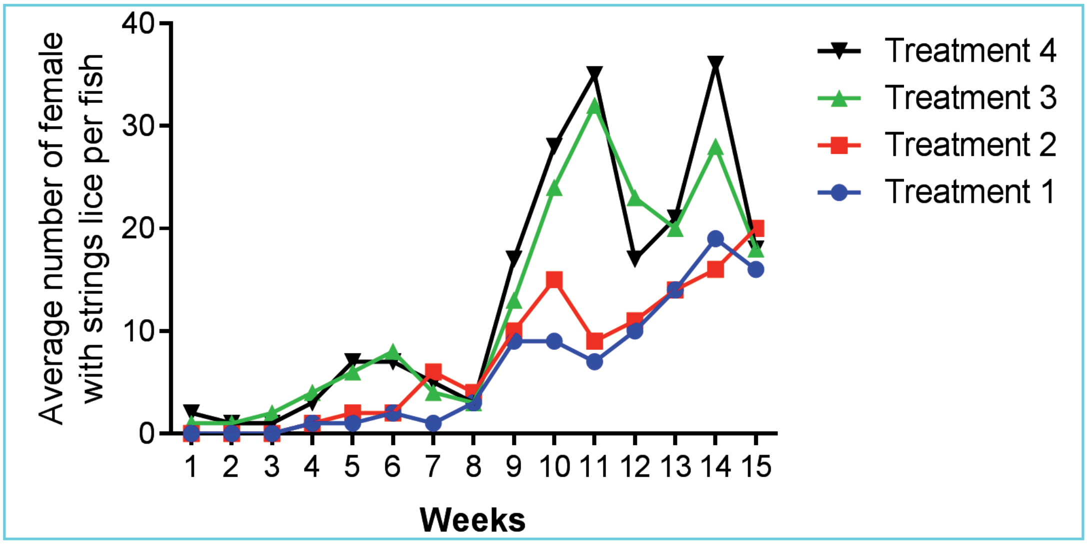

Fig. 24.6 An assessment of cleaning efficiency of the Patagonian blenny with salmon of different weights (Trial 5). Average number of female with strings of *Caligus rogercresseyi* per fish recorded for each duplicate treatment during each sampling date in Trial 5. Treatment 1: blenny:salmon ratio = 10% = 150 Atlantic salmon (initial weight 500 g) + 30 blenny (70 g); Treatment 2: 150 Atlantic salmon (initial weight 500 g); Treatment 3: (blenny:salmon ratio = 20%) = 175 Atlantic salmon (initial weight 3200 g) + 35 blenny (70 g); Treatment 4: 175 Atlantic salmon (initial weight 3200 g).

The reduction in sea lice numbers was 47.2% in treatment 1 after 35 days, compared with only 27.3% in the larger fish in treatment 3 (Fig. 24.6). The numbers of adult sea lice attached to salmon of 500 g and 3200 g/fish were reduced after 5 weeks. The numbers of sea lice removed from Atlantic salmon of 500 g was higher. There was a general reduction of the grazing activity from week 5 to week 10. This reduction in cleaning efficiency was associated with consumption of organisms fouling the nets by blenny and confirmed by gut content analysis.

In Trial 6 the cleaning efficiency of Patagonian blenny when reared with Atlantic salmon at different blenny:salmon ratios was assessed under experimental conditions in sea cages. The treatments were:

- Treatment 1: (P. blenny:salmon ratio = 10%) = 220 Atlantic salmon (initial weight 120 g) + 22 blenny (initial weight 70 g)
- Treatment 2: (blenny:salmon ratio = 30%) = 220 Atlantic salmon (initial weight 120 g) + 66 blenny (initial weight 70 g)
- Treatment 3: 220 Atlantic salmon (initial weight 120 g)

There was a reduction in sea lice numbers of 40% by day 7 at the low stocking ratio, Treatment 1, and 33% at day 49 at the high ratio (Fig. 24.7). There were no clear differences in cleaning efficiency between experimental treatments and control (Treatment 3) due to low sea lice numbers (<3 per fish).

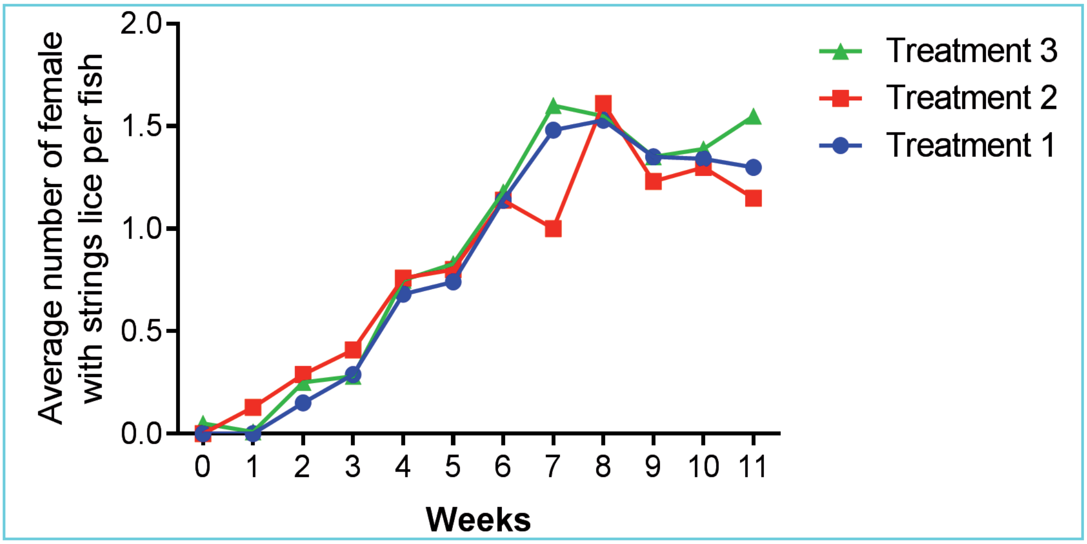

Fig. 24.7 The cleaning efficiency of Patagonian blenny when reared with Atlantic salmon at different blenny:salmon ratios under experimental conditions in sea cages. Data expressed as the average number of females with strings of *Caligus rogercresseyi* per fish recorded for each duplicate treatment during each sampling dates in the Trial 6. Treatment 1: (P. blenny:salmon ratio = 10%) with = 220 salmon (initial weight 120 g) + 22 blenny (initial weight 70 g); Treatment 2: (blenny:salmon ratio = 30%) = 220 salmon (initial weight 120 g) + 66 blenny (initial weight 70 g); Treatment 3: 220 salmon only (120 g).

From this series of six trials it is concluded that cleaning activity of the Patagonian blenny when reared with Atlantic salmon is evident under tank conditions, but is less clear in sea cages. The Patagonian blenny grazes on sea lice in the adult stage. Sea lice infection in the Patagonian blenny is significantly lower than that observed in Atlantic salmon. An increase in salmon stocking density and the P. blenny:salmon ratio showed a positive effect on cleaning efficiency under tank conditions. Blenny of weight range of 60 and <250 g consumed salmon feed sizes 4 mm and 6 mm. Higher cleaning efficiency was associated with Atlantic salmon of smaller size. Fouling of sea cage nets had a negative effect on cleaning activity of blenny.

24.4 Rearing of the Patagonian blenny

The Patagonian blenny has been successfully bred in captivity by personnel from Fundación Chile, at the Quillaipe Research Station. A brief description of this process is presented.

24.4.1 Broodstock conditioning

Wild-origin broodstock are captured during the autumn and kept in tanks maintained with a flow-through system for a period of four months. Fish are fed an extruded diet for salmonid broodstock. At the end of this period, gonad maturation is assessed.

24.4.2 Spawning, egg production and incubation

Spawning is induced using hormonal treatment through an intraperitoneal injection. After 72 hours, egg hydration takes place, and females show a pronounced belly enlargement. Eggs are then stripped after correct sedation of the fish. Immediately after spawning, eggs are fertilised by a gentle mixing with seminal fluid and water addition. Fertilised eggs are incubated in cylinder conical tanks. Egg segmentation starts one hour after fertilisation (Fig. 24.8) and extends for a

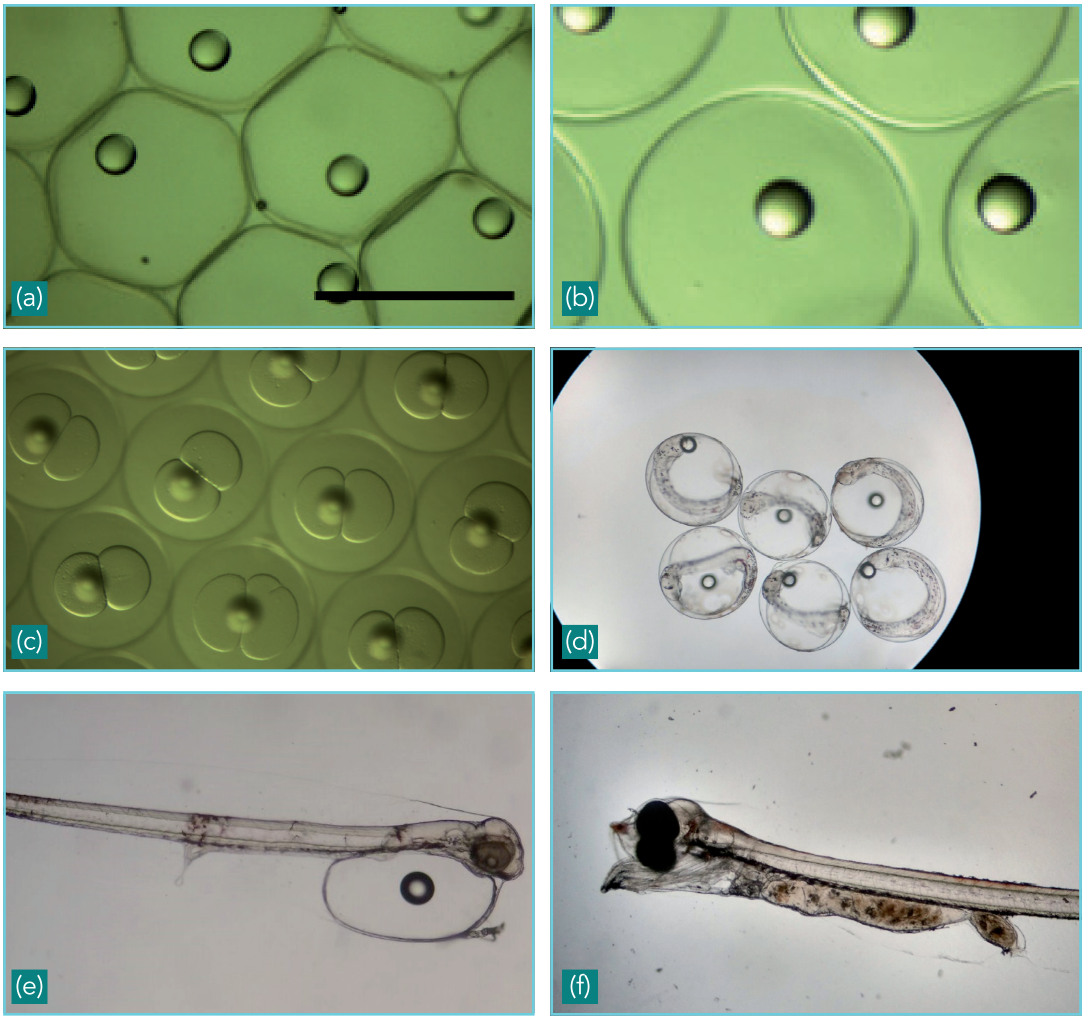

Fig. 24.8 Egg and larval developmental stages in Patagonian blenny. Clockwise from top left, Eggs (a) = unfertilised egg (scale ×56); Eggs (b) = 0.5 hours post-fertilisation (×56) 1.9 mm diameter; Eggs (c) = 3.5 hours post-fertilisation, first cleavage segmentation completed with two blastomeres (×56); Embryos (d) four days post-fertilisation (x4); Larva (e) one day post-hatching (×4), length of larvae 2.6 µm ; Larva (f) ten days post-hatch (×4). Credit: egg photos, Dr Ivan Valdebenito, Escuela de acuicultura, Universidad Católica de Temuco, Chile; embryo and larval photos, Mr Ramón Vidal, Fundación Chile, Estación Experimental Quillaipe, Puerto Montt, Chile.

period of nine hours. Hatching occurs 72 hours after fertilisation. Newly hatched larvae have an average length of 2.6 mm and, after ten days, the eyes are completely developed, as are the heart, digestive tract, mouth and pectoral fins. Enriched rotifers (*Brachionus plicatilis*) are used from first feeding to 20 days post-hatch (dph). Artemia metanauplii are incorporated from 20 dph. Live feed is gradually replaced with dry formulated feed from 40 dph.

A growth trial conducted with Patagonian blenny (initial weight = 40 g), indicated that maximum growth and nitrogen retention were obtained with 30% of dietary protein content. Optimum growth rate (expressed as specific growth rate) obtained in this trial was 0.58 (Sa *et al.*, 2014).

The hatch rate in the 2015 production season was 23% and a total of 4000 juveniles was produced. Patagonian blenny were transported by Fundación Chile in a tank with oxygen supply to the experimental cages. However, as this will be done on a commercial scale, fish should be transported by wellboat.

24.5 Future developments

Continuing work with the Patagonian blenny will include an evaluation of the effect of blenny body weight on lice cleaning efficiency, the evaluation of the effect of P. blenny origin (wild or farmed) on cleaning efficiency, the determination of blenny body size that can be stocked in sea cages, and the development of a model to determine the number of blenny to be used depending on factors such as season, farming zone and the salmon production phase, among others. Two other fish species have also been assessed as potential cleaner fish. These species include *Malapterus reticulatus* and *Mugil cephalus*. However, the results of these trials involving these species are still confidential. The industry in Chile hopes to be using cleaner fish on commercial salmon sea farms in the coming years (SalmonExpert, 2015).

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