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Short Communication

Coinfection of *Caligus lalandei* and *Benedenia seriola* on the yellowtail kingfish *Seriola lalandi* farmed in a net cage in northern Chile

Sandra Bravo¹, Carlos F. Hurtado² & María Teresa Silva³

¹Universidad Austral de Chile, Puerto Montt, Chile

²Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

³Universidad San Sebastián, Puerto Montt, Chile

Corresponding author: Sandra Bravo (sbravo@uach.cl)

ABSTRACT. The yellowtail kingfish *Seriola lalandi* is one of the most important fish candidates for the diversification of aquaculture in Chile. Fish farmed experimentally in one floating cage on a site located in Northern Chile, between November 2013 and December 2015, were first detected with the sea lice *Caligus lalandei* from April 2014. The highest prevalence (100%) was reached in July 2014 and the highest abundance in June 2014 (4.3 lice/fish). The monogenean *Benedenia seriola* was also recorded in the same stock of fish from July 2014, reaching the highest abundance of 30.8 parasite/fish and a prevalence of 100% in January 2015. When the abundance of *B. seriola* increased, *C. lalandei* abundance decreased until it disappeared in January 2015, which could be attributed to the increase of the seawater temperature in the period of study. This is the first report of *C. lalandei* infecting *S. lalandi* reared in net cage in Chile.

Keywords: *Seriola lalandi*, *Caligus lalandei*, *Benedenia seriola*, sea lice, monogenean ectoparasite, farmed kingfish.

The wild yellowtail kingfish *Seriola lalandi* has a migratory behaviour, with a world distribution covering Australia, New Zealand, Japan (Nugroho *et al.*, 2001), South East China Sea (Randall & Lim, 2000), the Mediterranean Sea (Nakada, 2008) and the Pacific coast from Canada to Chile (Eschmeyer & Herald, 1999; Dyer & Westneat, 2010; Fernández *et al.*, 2015). This wild species is annually recorded between November and April in northern Chile (20°S-30°S). (Sepúlveda & González, 2015a, 2015b). Commercial aquaculture production of yellowtail kingfish reared in net pen has been successfully established in Japan (Nakada, 2002), Australia (Hutson *et al.*, 2007) and New Zealand (Moran *et al.*, 2008), while in northern Chile, *S. lalandi* has been reared in the hatchery under controlled conditions since 2008.

Metazoan parasites have been shown to be an important threat for the yellowtail kingfish. The monogeneans *Zeuxapta seriola* and *Benedenia seriola* have been reported as the main parasitic problems of farmed kingfish (Ogawa & Yokoyama, 1998; Montero *et al.*, 2004; Hutson *et al.*, 2007). These flatworms can cause reduced appetite in heavy infections, slower growth

and, in extreme cases, can cause death to the host if left untreated (Sharp *et al.*, 2003; Whittington *et al.*, 2011). *B. seriola* is considered a major pathogen of farmed *S. lalandi* in South Australia and the major barrier to efficient production and industry growth (Chambers & Ernst, 2005; Lackenby *et al.*, 2007), while in Japan it is estimated that *B. seriola* is responsible for up to 22% of total production costs for the farmed Japanese yellowtail (*Seriola quinqueradiata*) (Ernst *et al.*, 2002). In contrast, the copepod *Caligus lalandei* has been reported in New Zealand (Jones, 1998; Sharp *et al.*, 2003) and in Japan infecting farmed yellowtail amberjack without serious problems (Ho *et al.*, 2001). In Chile, the monogenean *Zeuxapta seriola*, infecting the gill filament, and the copepod *C. lalandei* infecting the corporal surface have been reported on wild *S. lalandi* (Sepúlveda & González, 2015a, 2015b; Sepúlveda *et al.*, 2016).

The aim of this study is to describe the coinfection of the sea lice *C. lalandei* and *B. seriola* in a stock of *S. lalandi* reared for the first time in a sea cage in northern Chile (26°34'17"S, 70°41'29"W). In November 2013, 2,057 fish with a mean weight of 400 g, produced

Table 1. Prevalence, mean intensity and mean abundance of *Caligus lalandei* in the kingfish *Seriola lalandei*, per month and stage of development. n: number of sampled fish, SD: standard deviation, P: P-value, *No information was recorded.

Date	T°C	n	Prevalence (%)			Intensity			Abundance		
			Adult	Juvenile	Total	Z _c	P	Total Mean ± SD	Z _c	P	Total Mean ± SD
			Male	Female		Male	Female	Male	Female	Male	Female
2014 Jun	13.7	20	88.6	88.6	97.7	-	-	2.3	2.1	1.4	4.4 ± 2.3
Jul	13.6	20	41.2	76.5	100.0	0.7	0.25	1.6	1.8	1.0	2.4 ± 1.4
Aug*	13.7	-	-	-	-	-	-	-	-	-	-
Sep	13.8	20	58.6	51.7	69.0	3.0	0.00	1.3	2.0	1.4	3.2 ± 3.1
Oct.	13.7	20	56.0	60.0	0.0	0.5	0.31	1.9	1.7	0.0	2.7 ± 1.8
Nov	14.4	20	53.7	56.1	61.0	1.0	0.15	1.3	1.3	0.0	2.4 ± 1.4
Dec	14.6	20	6.3	18.8	21.9	2.7	0.00	1.5	1.7	0.0	1.9 ± 7.4
2015 Jan	15.6	20	0.0	0.0	0.0	2.4	0.01	0.0	0.0	0.0	0.0
Feb	15.3	20	0.0	0.0	0.0	0	0	0.0	0.0	0.0	0.0

in captivity under controlled conditions in a hatchery with a recirculating system, were transferred to a circular submersible cage made with copper alloy mesh (20 m diameter and 10 m depth). When fish reached a mean weight of 1,250 g in April 2014, the ectoparasite *C. lalandei* was recorded infesting farmed fish for the first time in a routine sample weight. After that finding, 20 fish were monitored monthly to evaluate the abundance of parasites in the fish population (Table 1). Prevalence was calculated as the percentage of infected fish in the sample, mean abundance as the mean number of parasites per fish examined, and the mean intensity as the mean number of parasites per infected fish (Rózsa *et al.*, 2000).

Fish were collected with a hand net, and then anaesthetised with benzocaine (10% in ethanol, 1 mL⁻¹) prior to individual examination by eye. Parasites were counted and classified according to gender and stage of development (female, male and juvenile), and fish were returned carefully to the same cage after sampling. Parasites were stored in 95% ethanol for later examination, and subsequently identified according to morphological features.

The identity of *C. lalandei* (Fig. 1) was confirmed by comparison with the paratypes of *C. lalandei* in the collections of The Natural History Museum, London, while the identity of *B. seriolae* (Fig. 2) was confirmed by consulted bibliography (Baeza & Castro, 1975; Kearn *et al.*, 1992; Kinami *et al.*, 2005). The Z test for significant differences of proportion was used to assess differences in prevalence between months, and the *t*-test to assess differences in abundance and intensity between months.

In the period of study, the temperature ranged between 13.6 (July 2014) and 15.3°C (February 2015), and salinity ranged between 34.3 and 34.7. The highest prevalence of *C. lalandei* was recorded in July 2014 (100%), when *B. seriolae* was recorded infecting the same stock of fish. Since that date, the prevalence of *C. lalandei* decreased to 0% in January 2015, while the prevalence of *B. seriolae* increased from 5.9% in July to 100% in January 2015 (Fig. 3).

No data were collected in August 2014 because of winter storm conditions. The highest abundance and intensity of infection of *C. lalandei* was recorded in June 2014 (Figs. 4-5) when the seawater temperature reached 13.7°C, decreasing through the months to reach an abundance of 1.1 lice/fish and an intensity of infection of 1.9 lice/fish in December 2014 when the mean temperature was 14.6°C (Table 1). No *Caligus* were recorded in January and February, while the monogenean *B. seriolae* was recorded from July 2014 with an abundance of 0.1 parasite/fish (Table 2). In contrast to *C. lalandei*, the abundance of *B. seriolae*



Figure 1. *Seriola lalandi* infected with gravid female of *Caligus lalandei* (Arrow).



Figure 2. *Seriola lalandi* infected with *Benedenia seriola* (Arrows).

increased through the following months to reach 30.8 parasites/fish in January 2015, when temperature reached 15.6°C (Fig. 4). There was no mortality or damage to the skin associated with the infestation of *C. lalandei* during the period of study, nor to *B. seriola*. Therefore no treatment was required to control these parasites on fish kept at a density lower than 1 kg m⁻³. This farm experiment was completed when fish reached an average weight of 2.55 kg in February 2015.

Although *S. lalandi* has been reared in captivity in northern Chile since 2008 no parasites were previously reported in farmed fish as this species was only reared under controlled conditions in a hatchery with a recirculating system. The infestation recorded on farmed *S. lalandi* in sea cage by *C. lalandei* and *B. seriola*, can be explained by the presence of the wild kingfish *S. lalandi* in the area where the cage was settled (*pers. observ.*). In fact *B. seriola* was reported infecting the wild kingfish *S. lalandi* in northern Chile

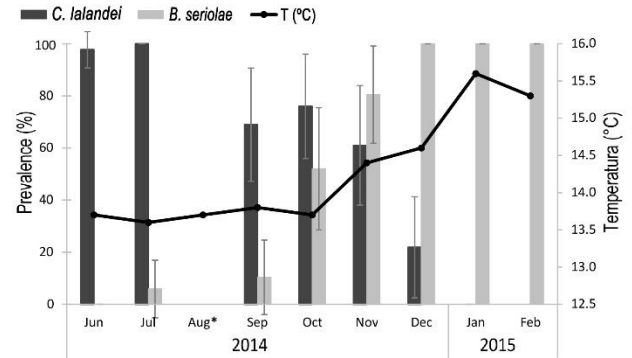


Figure 3. Monthly prevalence and confidence interval 95% of *Caligus lalandei* and *Benedenia seriola* in the kingfish *Seriola lalandi* in the period of study, in relation to the seawater temperature. No information was recorded in August 2014.

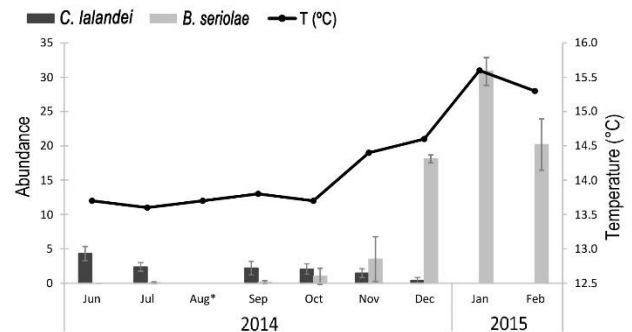


Figure 4. Mean abundance and confidence interval 95% of *Caligus lalandei* and *Benedenia seriola* infecting the kingfish *Seriola lalandi* in the period of study, in relation to the seawater temperature. No information was recorded in August 2014.

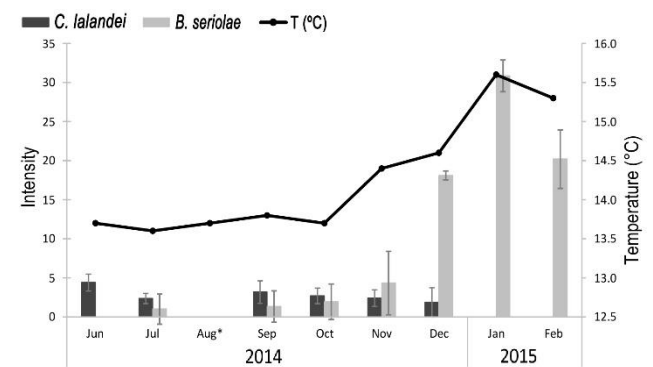


Figure 5. Mean intensity and confidence interval 95% of *Caligus lalandei* and *Benedenia seriola* infecting the kingfish *Seriola lalandi* in the period of study, in relation to the seawater temperature. No information was recorded in August 2014.

for the first time in 1975 (Baeza & Castro, 1975) and *C. lalandei* in 1980 (Baeza & Castro, 1980). A similar situation was reported in Australia, where fingerlings

Table 2. Prevalence, mean intensity and mean abundance of *Benedenia seriola* in the kingfish *Seriola lalandi*, per month and stage of development. n: number of sampled fish, SD: standard deviation. *No information was recorded.

Date	T°C	n	Prevalence (%)			Intensity			Abundance		
			Adult	Juvenile	Total	Z _c	P	Adult	Juvenile	Mean ± SD	P
2014 Jun	13.7	20	0.0	0.0	0.0	-	-	0.0	0.0	0.0	-
Jul	13.6	20	5.9	0.0	5.9	1.1	0.13	ns	0.0	0.1 ± 0.2	1.8 0.03 s
Aug*	13.7		-	-	-	-	-	-	-	-	-
Sep	13.8	20	10.3	0.0	10.3	0.5	0.30	ns	0.0	0.1 ± 0.4	0.0 0.50 ns
Oct.	13.7	20	52.0	0.0	52.0	3.2	0.00	s	0.0	1.0 ± 2.5	1.6 0.06 ns
Nov	14.4	20	73.2	34.1	80.5	2.0	0.02	s	3.6	3.5 ± 6.9	1.5 0.07 ns
Dec	14.6	20	100.0	93.8	100.0	2.2	0.01	s	7.4	18.1 ± 1.2	9.3 0.00 s
2015 Jan	15.6	20	100.0	100.0	100.0			5.2	25.6	30.8 ± 4.3	12.7 0.00 s
Feb	15.3	20	100.0	95.2	100.0			15.9	4.3	20.2 ± 7.9	

reared in land-based hatcheries were parasite-free but, when they were transferred to sea-cages for grow out, fluke populations proliferated due to their direct life cycle and the reservoir of infections on wild *S. lalandi* (Whittington *et al.*, 2011).

B. seriola is a cosmopolitan species reported as the most pernicious parasite of farmed kingfish around the world (Ogawa & Yokoyama, 1998; Whittington *et al.*, 2001; Tubbs *et al.*, 2005). Therefore medicinal treatments such as freshwater baths and hydrogen peroxide are used for its control (Chambers & Ernst, 2005). Although no mortality was associated with this parasite in this study, a reduction in growth was recorded since December 2015, which could be associated in part to the *Benedenia* infestation, such as has been reported by other authors (Whittington, 2005; Whittington & Chisholm, 2008).

C. lalandei is also considered a cosmopolitan species reported for the first time from wild *S. lalandi* caught in Kalk Bay, South Africa (Barnard, 1948). Subsequently, it was reported from the same wild fish species in Chile (Baeza & Castro, 1980) and New Zealand (Jones, 1998; Sharp *et al.*, 2003). *C. lalandei* infecting farmed yellowtail was reported for the first time in Japan without serious problem associated with disease, although Ho *et al.* (2001) suggested it may cause a serious problem in the event of an outbreak because of its large size in comparison with other *Caligus* species.

In this study, fish did not show physical damage that would require any medicinal intervention to control both parasite species, perhaps because fish were reared at a density that did not exceed 1 kg m⁻³ in the experimental sea-cage. The most relevant issue was that the higher prevalence and abundance of *C. lalandei* was recorded in the winter period, declining until it was absent in the summer months (Table 1), when the prevalence and abundance of *B. seriola* increased (Table 2), which seems to be associated with the seawater temperature (Figs. 3-4). In fact, Ernst *et al.* (2005) demonstrated that temperature strongly influenced the embryonic period in *B. seriola*. At high temperature the eggs hatch earlier than at low temperature, which can explain the increased abundance in the summer period. In contrast, there is no antecedent that could explain the declining abundance of *C. lalandei* in the same period.

This situation will be examined in further studies to understand the epidemiological behaviour of both parasites, and the factors affecting the prevalence and abundance on *S. lalandi*. This will enable procedures and disease management strategies to be developed, in order to minimize future losses during the rearing period in sea cages.

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