***Short Communication***

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

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**ABSTRACT.** The wild 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 *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 seriolae* 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. seriolae* increased, *C. lalandei* abundance decreased until it disappeared in January 2015, which could be attribute to the increase of the seawater temperature in the period of study. This is the first report of *Caligus lalandei* infecting *Seriola lalandi* reared in net cage in Chile.

**Keywords**: farmed kingfish; *Seriola lalandi*; sea lice; *Caligus lalandei*; *Benedenia seriolae*

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 in the summer season in northern Chile (20ºS - 30ºS) (Sepulveda & 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 rearing in hatchery under controlled conditions since 2008.

Metazoan parasites have been shown to be an important threat for the yellowtail kingfish. The monogeneans *Zeuxapta seriolae* and *Benedenia seriolae* have been reported as the main parasitic problems of farmed kingfish (Ogawa and 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). Benedenia seriolae is considered a major pathogen of farmed *S. lalandi* in South Australia and the major barrier to efficient production and industry growth (Chambers and Ernst, 2005; Lackenby *et al*., 2007), while in Japan it is estimated that *B. seriolae* is responsible for up to 22% of total production costs for the farmed Japanese yellowtail (Seriola *quinqueradiata)* (Ernst *et al.,* 2002). By other way, 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 seriolae*, infecting the gill filament, and the copepod *C. lalandei* infecting the corporal surface have been reported on wild *S. lalandi* (Sepulveda & González 2014, 2015; Sepúlveda *et al*., 2016).

The aim of this study is to describe the coinfection of *Caligus lalandei* and Benedenia seriolae in a stock of *S. lalandi* reared by first time in a sea cage in northern Chile (26º34'17"S, 70º41'29"W). In November 2013, 2,057 fish with a mean weight of 400 g, produced in captivity under controlled conditions in a hatchery with a recirculating system, were transferred to a circular submersible cage made with cooper alloy mesh (20 m diameter and 10 m depth). When fish reached a mean weight of 1,250 g in April 2014, the ectoparasite *Caligus lalandei* was by first time recorded infesting the farmed fish. After that finding, 20 fish were monthly monitored to evaluate the abundance of lice on 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).

Collected fish were anesthetized with benzocaine (10% in ethanol, 1 ml L− 1) and each fish examined macroscopically. Lice were classified according to sex and stage of development (female, male and juvenile). In the period of study the temperature ranged between 13.6 (July 2014) and 15.3ºC (February 2015), and salinity ranged between 34.3 and 34.7 ppt.

The highest prevalence of *C. lalandei* was recorded in July 2014 (100%), when *Benedenia 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. 1). No data were collected in August 2014 because of bad weather conditions (Table 1). The highest abundance and intensity of infection of *C. lalandei* was recorded in June 2014 (Fig. 2; Fig.3) when the seawater temperature reached 13.7ºC, decreasing through the months to reach in December 2014 an abundance of 1.1 lice/fish and an intensity of infection of 1.9 lice/fish when the temperature was a mean of 14.6ºC (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. In contrast to *C. lalandei*, the abundance of *B. seriolae* increased through the following months to reach 30.8 parasites/fish in January 2015, when temperature reached 15.6ºC (Table 1). There was no mortality or damage to the skin associated with the infestation of *C. lalandei* during the period of study, nor to *B. seriolae*. Therefore no treatment was required to control these parasites on fish kept in density lower than 1 kg/m3. 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. seriolae,* can be explained by the presence of the wild kingfish *S. lalandi* in the area where the cage was settled (personal observation). In fact *B. seriolae* was reported infecting the wild kingfish *S. lalandi* in northern Chile 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 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. seriolae* is a cosmopolitan species reported as the most pernicious parasite of farmed kingfish around the world (Ogawa & Yokoyama, 1998; Tubbs *et al*., 2005; Whittington *et al*., 2001). Therefore medicinal treatments such as freshwater baths and hydrogen peroxide are used for its control (Chambers & Ernst, 2005). Although not mortality was associated to this parasite in this study, reduction growth was recorded since December 2015, which could be associated in part to the *Benedenia* infestation, such as it as 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 by 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 to 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 rearing in density that not exceeded 1 kg/m3 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, when the prevalence and abundance of *B. seriolae* increased, which seems to be strongly associated to the seawater temperature (Table 1). This situation will be examined in further studies to understand the epidemiological behaviour of both parasites, and the factors affecting its prevalence and abundance on *S. lalandi.* This will enable procedures and disease management strategies to be developed, and in this way to minimize future losses during the rearing period in sea cages.

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**REFERENCES**

Baeza, H. & R. Castro. 1975.*Benedenia melleni* (MacCallum, 1927) ectoparásito en peces de Antofagasta (Platyhelminthes, Monogenea). Apuntes Oceanológicos 7: 14-22.

Baeza, H. & R. Castro. 1980. Tres especies de *Caligidae* nuevas para la fauna chilena (Copepoda, Siphonostomatoida). Not. Mens. Mus. Nac. Hist. Nat., 25, 3–7

Barnard, K.H. 1948. New records and descriptions of new species of parasitic Copepoda of South Africa. Ann & Mag. Nat. Hist. Ser., 12 1, 242–254

Chambers, C. & I. Ernst. 2005. Dispersal of the skin fluke *Benedenia seriolae* (Monogenea: Capsalidae) by tidal currents and implications for sea-cage farming of *Seriola* spp. Aquaculture 250, 60–69.

Dyer, B.S. & M.W. Westneat. 2010. Taxonomy and biogeography of the coastal fishes of Juan Fernández Archipelago and Desventuradas Islands, Chile. Rev. Biol. Mar. Oceanogr. 45: 589-617.

Ernst, I., Whittington, I., Corneillie, S. & C. Talbot. 2002. Monogenean parasites in sea-cage aquaculture. Austasia Aquacult., 16, 46–48.

Eschmeyer, W.N. & E.S. Herald. 1999. A field guide to Pacific coast fishes: North America. Houghton, Mifflin, New York, 352 pp.

Fernández, G., Cichero, D., Patel A. & V. Martínez. 2015. Genetic structure of Chilean populations of *Seriola lalandi* for the diversification of the national aquaculture in the north of Chile. Lat. Am. J. Aquat. Res., 43 (2):374-379.

Ho, J.S., Nagasawa, K., Kim I.H. & K. Ogawa. 2001. Occurrence of *Caligus lalandei* Barnard, 1948 (Copepoda, Siphonostomatoida) on amberjacks (*Seriola* spp.) in the western North Pacific. Zool. Sci*.* 18: 423-431.

Hutson, K.S., Ernst, I. & I.D. Whittington. 2007. Risk assessment for metazoan parasites of yellowtail kingfish *Seriola lalandi* (Perciformes: Carangidae) in South Australian sea-cage aquaculture. Aquaculture 271: 85-99.

Jones, B. 1998. New Zealand parasitic Copepoda; genus *Caligus* Muller, 1785 (Siphonostomatoida: Caligidae). N. Z. J. Zool.*,* 15: 397–413.

Lackenby, J.A., Chambers, C.B., Ernst I. & I.D. Whittington. 2007. Effect of water temperature on reproductive development of *Benedenia seriolae* (Monogenea: Capsalidae) from *Seriola lalandi* in Australia. Dis. Aquatic Organisms. 74: 235–242.

Montero, F. E., Crespo, S., Padrớs, F., De la Gándara, F., García, F. A. & J.A. Raga. 2004. Effects of the gill parasite *Zeuxapta seriolae* (Monogenea: Heteraxinidae) on the amberjack *Seriola dumerili* Risso (Teleostei: Carangidae) Aquaculture*,* 232: 153-163.

Moran, D., Wells, R.M. & S.J. Pether. 2008. Low stress response exhibited by juvenile yellowtail kingfish (Seriola lalandi Valenciennes) exposed to hypercapnic conditions associated with transportation. Aquacult. Res., 39: 1399-1407.

Nakada, M. 2002. Yellowtail culture development and solutions for the future. Rev. Fish Sci*.,* 10: 559-575.

Nugroho, E., Ferrell, D.J., Smith, P. & N. Taniguchi. 2001. Genetic divergence of kingfish from Japan, Australia and New Zealand inferred by microsatellite DNA and mitochondrial DNA control region markers. Fish. Sci., 67: 843-850.

Ogawa, K. & H. Yokoyama. 1998. Parasitic diseases of cultured marine fish in Japan. Fish Pathol., 33(4): 303–309.

Randall, J.E. & K.K.P. Lim. 2000. A checklist of the fishes of the South East China Sea. Raffles. Bull. Zool., S8: 569-667.

Rózsa, L., Reiczigel, J. & G. Majoros. 2000. Quantifying parasites in samples of hosts. J. Parasitol., 86(2): 228–232

Sepulveda, F.A., Torres, J.F., Infante C.D. & M.T. González. 2016. Potential role of ectoparasites (*Zeuxapta seriolae* and *Caligus lalandei*) in the transmission of pathogenic bacteria in yellowtail kingfish *Seriola lalandi*, inferred from cultivable microbiota and molecular analyses. J. Fish Dis. doi:10.1111/jfd.12582.

Sepulveda, F.A. & M.T. González. 2015a. Patterns of genetic variation and life history traits of *Zeuxapta seriolae* infesting *Seriola lalandi* across the coastal and oceanic areas in the south eastern Pacific Ocean: potential implications for aquaculture. Parasites vectors, 8: 282. DOI 10.1186/s13071-015-0892-4.

Sepulveda, F.A. & M.T. González. 2015b. Molecular and morphological analyses reveal that the pathogen *Benedenia seriolae* (Monogenea: Capsalidae) is a complex species: Implications for yellowtail Seriola spp. Aquaculture, 418–419 (2014): 94–100.

Sharp, N. J., Poortenaar, C. W., Diggles, B. K. & T.J. Willis. 2003. Metazoan parasites of yellowtail kingfish, *Seriola lalandi lalandi*, in New Zealand: prevalence, intensity, and site preference. New Zealand Journal of Marine and Freshw. Res., 37: 273–282.

Tubbs, L., Poortenaar, C., Sewell, M. & B. Diggles. 2005. Effects of temperature on fecundity in vitro, egg hatching and reproductive development of *Benedenia seriolae* and *Zeuxapta seriolae* (Monogenea) parasitic on yellowtail kingfish *Seriolae lalandi*. Int. J. Parasitol. 35, 315–327.

Whittington, I.D., Shinn, A.P., Bron, J.E. & M.R. Deveney. 2011. Innovative Solutions for Aquaculture: Assessment of *in situ*monitoring techniques and life history parameters for monogenean skin and gill parasites. Final Report to FRDC (Project No. 2003/221). The University of Adelaide, Adelaide, South Australia. 41 pp.

Whittington, I.D. 2005. Monogenea Monopisthocotylea (ectoparasitic flukes). In: Marine Parasitology. Rohde K. (ed.). CSIRO Publishing, Melbourne, Australia, pp. 63-72.

Whittington, I.D. & L.A. Chisholm. 2008. Diseases caused by Monogenea. In: Fish Diseases Eiras J.C., Segner H., Wahlii T. & Kapoor B.G. (eds.). Science Publishers, Inc., New Hampshire, USA. Vol. 2. (Chapter 13) pp. 683-816.

Whittington, I.D., Corneillie, S., Talbot, C., Morgan, J. & R. Adlard. 2001. Infections of Seriola quinqueradiata Temminck & Schlegel and S. dumerili (Risso) in Japan by Benedenia seriolae (Monogenea) confirmed by morphology and 28S ribosomal DNA analysis. J. Fish Dis., 24: 421–425.



**Figure 1:** Monthlyprevalence and confidence interval 95% for *Caligus lalandei* and *Benedenia seriolae* in the kingfish *Seriola lalandei* in the period of study. Not information was recorded in August 2014.



**Figure 2:** Mean abundance and confidence interval 95% for *Caligus lalandei* and *Benedenia seriolae* infecting the kingfish *Seriola lalandei* in the period of study. Not information was recorded in August 2014.



**Figure 3:** Mean intensity and confidence interval 95% for *Caligus lalandei* and *Benedenia seriolae* infecting the kingfish *Seriola lalandei* in the period of study. Not information was recorded in August 2014.

**Table 1:** Prevalence, mean intensity and mean abundance of *Caligus lalandei* and *Benedenia seriolae* in the kingfish *Seriola lalandei,* per month and stage of development.



\* not information was recorded