Preventive Veterinary Medicine 153 (2018) 7–14

Contents lists available at ScienceDirect

Preventive Veterinary Medicine

journal homepage: www.elsevier.com/locate/prevetmed

Random and systematic sampling error when hooking fish to monitor skin fluke (*Benedenia seriolae*) and gill fluke (*Zeuxapta seriolae*) burden in Australian farmed yellowtail kingfish (*Seriola lalandi*)

J.R. Fensham[a,b], E. Bubner[c], T. D'Antignana[c], M. Landos[b], C.G.B. Caraguel[a,*]

[a] School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy Campus, Roseworthy, South Australia, 5371, Australia
[b] Future Fisheries Veterinary Services Pty Ltd, East Ballina, New South Wales, 2478, Australia
[c] Lincoln Marine Science Centre, School of Biological Sciences, Flinders University, Port Lincoln, South Australia, 5606, Australia

ARTICLE INFO

Keywords:
Seriola lalandi
Sampling bias
Sampling error

ABSTRACT

The Australian farmed yellowtail kingfish (*Seriola lalandi*, YTK) industry monitor skin fluke (*Benedenia seriolae*) and gill fluke (*Zeuxapta seriolae*) burden by pooling the fluke count of 10 hooked YTK. The random and systematic error of this sampling strategy was evaluated to assess potential impact on treatment decisions.

Fluke abundance (fluke count per fish) in a study cage (estimated 30,502 fish) was assessed five times using the current sampling protocol and its repeatability was estimated the repeatability coefficient (CR) and the coefficient of variation (CV). Individual body weight, fork length, fluke abundance, prevalence, intensity (fluke count per infested fish) and density (fluke count per Kg of fish) were compared between 100 hooked and 100 seined YTK (assumed representative of the entire population) to estimate potential selection bias.

Depending on the fluke species and age category, CR (expected difference in parasite count between 2 sampling iterations) ranged from 0.78 to 114 flukes per fish. Capturing YTK by hooking increased the selection of fish of a weight and length in the lowest 5th percentile of the cage (RR = 5.75, 95% CI: 2.06–16.03, *P*-value = 0.0001). These lower end YTK had on average an extra 31 juveniles and 6 adults *Z. seriolae* per Kg of fish and an extra 3 juvenile and 0.4 adult *B. seriolae* per Kg of fish, compared to the rest of the cage population (*P*-value < 0.05).

Hooking YTK on the edge of the study cage biases sampling towards the smallest and most heavily infested fish in the population, resulting in poor repeatability (more variability amongst sampled fish) and an overestimation of parasite burden in the population. In this particular commercial situation these finding supported that health management program, where the finding of an underestimation of parasite burden could provide a production impact on the study population. In instances where fish populations and parasite burdens are more homogenous, sampling error may be less severe. Sampling error when capturing fish from sea cage is difficult to predict. The amplitude and direction of this error should be investigated for a given cultured fish species across a range of parasite burden and fish profile scenarios.

1. Introduction

Ectoparasitic infestations represents substantial fish health and welfare challenges for sea cage aquaculture systems worldwide (Whittington et al., 2000; Ernst et al., 2002). Industry implications of such infestations include; direct stock loss, depressed fish growth, poor fish health and welfare, reduced value of market product, and costs associated with monitoring and treatment programmes (Sharp et al., 2003; Hutson et al., 2007; Ernst et al., 2002). In Port Lincoln, Australia, the yellowtail kingfish (*Seriola lalandi*, YTK) industry has suffered substantial production setbacks in recent years due to recurrent infestation of two monogenean ectoparasites; *Benedenia seriolae* (skin fluke, SF, Sub-class Monopisthocotylea) and gill fluke, *Zeuxapta seriolae* (gill fluke, GF, Sub-class Polyopisthocotylea) (Hutson, 2007). These two parasites have a direct lifecycle, with adult stages colonising and feeding on the fish and mature adult females releasing egg bundles that

Abbreviations: YTK, yellowtail kingfish; LE, low extreme fish (less than or equal to the 5th percentile weight or fork length of seined fish); N, Normal fish (weight and fork length greater than LE fish); HE, High extreme fish (greater than or equal to the 95th percentile weight or fork length of seined fish); CV, coefficient of variation; CR, coefficient of repeatability; FL, fork length; W, weight; RR, Relative Risk; GF, gill fluke (*Zeuxapta seriolae*); SF, skin fluke (*Benedenia seriolae*)

* Corresponding author.
E-mail address: charles.caraguel@adelaide.edu.au (C.G.B. Caraguel).

https://doi.org/10.1016/j.prevetmed.2018.02.012
Received 31 March 2017; Received in revised form 4 January 2018; Accepted 22 February 2018

attach on cage infrastructure allowing for rapid re-infestation and amplification in sea cage systems where fish hosts are stocked in high density (Tubbs et al., 2005). Both flukes are specific to the *Seriola* genus and do not represent any concern for human consumption (Hayward, 2005).

SF actively feed on epithelial cells following attachment to skin surfaces (Whittington, 2005) which cause skin irritation and depression in feed intake of infested host which respond by rubbing against the cage net and any floating devices. Subsequently skin lesions can occur with erosions and progressing to ulceration and secondary bacterial infections, in severe cases (Whittington, 2005; Ernst et al., 2002). GF are sanguineous, attaching exclusively to the gill lamellae resulting with time in anaemia, jaundice and emaciation of the fish host (Grau et al., 2003; Chih-Hui et al., 2012). Destruction of gill epithelium and vascular damage at the attachment site induces focal gill inflammation and lamellar fusion (Montero, 2004). The duration of the flukes' life cycle is temperature dependent and uncontrolled outbreaks commonly occur during summer months (Ernst et al., 2002). The increase in sea water temperature shortens the duration of fluke maturation, incubation period and increases egg hatchability (Tubbs et al., 2005).

The control of YTK flukes involves treating the sea cage population with a hydrogen peroxide bathe (Mansell et al., 2005). This process is costly, labour intensive, logistically complex and has narrow safety margins (Mansell et al., 2005; Williams et al., 2007). Hydrogen peroxide does not destroy fertilised fluke eggs (Sharp et al., 2004) and within few days to weeks (according to sea temperatures) a new generation of flukes hatches and reinfests the cage (Tubbs et al., 2005). Therefore, bathing strategy uses a second consecutive bath to timely kill the newly hatched juvenile flukes before they reach sexual maturity and release new eggs. The time lapse between bathing depends on water temperature and is dictated by the burden and age distribution of flukes in the cage. The monitoring of flukes' burden in the cages is instrumental to optimise bathing schedule (Whittington, 2005). Poorly timed treatments may waste resources (too early) or impact productivity, fish health and welfare (too late). The accuracy of the fluke monitoring is paramount to properly time treatment.

Following commercial reality, monitoring of parasite burden in aquaculture should be fit-for-purpose, i.e. providing accurate and meaningful management information for the least resources (time, labour, and money) possible (Revie et al., 2007). In Australia, the industry fluke monitoring protocol involves capturing up to ten fish using hook-and-line from the edge of the sea cage. This method of fish capture is routinely used in other aquaculture industries to conveniently sample fish. However, hook-and-line is believed to bias the sample, especially when the fish population is not homogenous (Oidtmann et al., 2013). Fish cage populations are rarely homogenous in size and growth because of the hierarchical nature of fish interaction (dominant fish grow faster and bigger). Parasite burden is also expected to not be uniform especially at the early stage of the colonisation when not all the fish are infested (Heuch et al., 2011). It was expected that large and dominant fish are preferentially sampled using hook-and-line (lure-based method), and that also larger fish are healthier. In consequence, low parasitized fish would be over-represented in the sample and the parasite burden in the cage would be under-estimated. An under-estimation of fluke burden in YTK cage would delay treatment and potentially allow the next generation flukes to reach sexual maturity and release eggs in the environment before intervention. The knowledge of the presence and direction of a sampling error when using hook-and-line was deemed of primary importance by the Australian YTK farming industry to properly schedule fluke treatments.

The aim of this study was to evaluate the presence of random and systematic sampling error of the SF and GF burden monitoring in sea caged YTK. The objectives were to evaluate; firstly, the repeatability (precision) of the industry protocol and, secondly, the potential of hook-and-line sampling to bias the estimate of fluke burden. It was hypothesised that hook-and-line biases towards larger, less parasitized YTK and therefore underestimates fluke burden in the sea cage population.

2. Materials and methods

2.1. Study population

The study site was a commercial yellowtail kingfish (YTK, *Seriola lalandi*) farm in Boston Bay, offshore of Port Lincoln (South Australia) experiencing chronic infestation with *Z. seriolae* (GF) and *B. seriolae* (SF). A single 40 m diameter sea cage of approximately 30,000 YTK was sampled over two consecutive days (23rd and 24thJune 2014; sea temperature 15.7 °C). The study cage was previously treated for flukes on 8th April 2014, 11 weeks before sampling, using a hydrogen peroxide bathe (186 mg L^{-1} for 24 min), and was previously graded 4–6 weeks prior to the study.

2.2. Repeatability of the industry protocol

On the first study day and according to industry protocol, a pool of 10 fish were captured from the edge of the sea cage using a hook-and-line method and transferred into a 1000 L anaesthetic bath of 8.5 mg L^{-1} AQUI-S® (iso-eugenol) for 7–10 min, until complete anaesthesia was achieved (as described by Sharp et al., 2004). Anaesthetised fish were visually inspected for juvenile and adult SF (visual individual count) before transfer into a 200L tank containing seawater and praziquantel (5 mg L^{-1} for 10 min) to primarily dislodge adult and juvenile GF (Mansell et al., 2005; Mooney et al., 2006). Bathe solution was filtered through a 40 µm size mesh sieve to collect dislodged flukes into 70 mL screw top plastic sample containers for subsequent pooled microscopic count. Following the praziquantel bathe, fish were returned to a 1000 L recovery bath containing clear seawater and, upon full recovery, the caudal fin was clipped, and the fish released back into the sea cage. This protocol was repeated five times in succession to evaluate the repeatability of the method (total of 50 fish sampled).

2.3. Sampling bias of hooking fish

On the same first study day, 100 fish were captured by hook-and-line method in series of 10–20 fish at the time from the edge of the cage. Captured fish were anaesthetised as described above. Anaesthetised fish were weighed, measured and visually inspected to count juvenile and adult SF before transfer into individual black coloured 52 L plastic tubs containing seawater and praziquantel (5 mg L^{-1} for 10 min) to dislodge GF. Next, fish were transferred directly into a second individual 52 L black plastic tub containing clear freshwater for 10 min to dislodge SF. Afterward, fish were visually inspected a second time to count any remaining juvenile and adult SF before transfer into a 1000 L harvest bin with seawater to fully recover from the anaesthetic. Upon recovery, the fish caudal fin were clipped before return to the cage. The praziquantel and freshwater baths were filtered through the same 40 µm sieve to collect dislodged flukes into a 70 mL screw top plastic sample containers for subsequent individual fish microscopic count.

On the second study day, approximately half of the same sea cage was crowded into a homogeneous mix (no discriminative swimming behaviour possible) using a large harvest seine net and 100 fish were captured using a wet harvest brail. The fish were transferred into a 1000 L harvest bin containing seawater and a lighter dose of AQUI-S® (4 mg L^{-1}) to be tranquilised until sampling. When required, a few fish were transferred into another 1000 L harvest bin containing seawater and an anaesthetic dose of AQUI-S® (8.5 mg L^{-1}) to be anaesthetised. Anaesthetised fish were weighed, measured, visually assessed and individually processed for individual collection of flukes as described previously for the 100 hooked fish.