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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*)

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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.

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*)

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) (Clean Seas Tuna Ltd, 2012/13). These two parasites have a direct lifecycle, with adult stages colonising and feeding on the fish and mature adult females releasing egg bundles that 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

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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 ressources (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

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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 metre diameter sea cage of approximately 30,000 YTK was sampled over two consecutive days (23rd and 24thJune 2014; sea temperature 15.7^{o}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 1,000 L anaesthetic bath of 8.5 mg L^{-1} AQUI-S® (iso-eugenol) for 7–10 minutes, 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 1,000 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 1,000 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 1,000 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 1,000 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.

2.4 Parasite counting

Visual SF counts were performed during field sampling with adults (large size flukes) found on the body of the YTK and juveniles (small size flukes) around and on the YTK eyes. Flukes collected into 70 ml seawater jars were fixed later in the laboratory in 70% ethanol (v/v) and shelved until counting. For counting, each sample jar was emptied on a plastic petri dish scored at the bottom with seven parallel lines 1cm apart. Fixed flukes were identified and counted with manual tally counters as SF or GF and as either adult or juvenile under a dissecting microscope (at 10–20x magnification). Adult SF were differentiated from juvenile

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by the presence of a central chamber containing oocytes and a length greater than 3.7 mm (Tubbs et al., 2005; Mooney et al., 2006). Adult GF were differentiated from juvenile by the presence of vitellarium, a central yolk duct, and longitudinal hemosiderin pigmentation (Tubbs et al., 2005; Mooney et al., 2006).

2.5 Data handling and analysis

All data was entered and formatted in MS Excel 2007 and analysed using the statistical package STATA v.13.1 (StataCorp LP, Texas, USA).

2.5.1 Repeatability of the industry protocol

The precision of the industry fluke monitoring protocol was evaluated using the repeatability coefficient (CR), i.e. value below which the absolute differences between two measures would lie with 95% probability (Vaz et al., 2013) and coefficient of variation (CV), i.e. relative variability (%) between two repeated measures (Shechtman, 2013):

$$Repeatability\ coefficient\ (CR) = 2 \times \sqrt{2 \times \hat{\sigma}^2} \qquad (1)$$

$$Coefficient\ of\ variation\ (CV) = \frac{\hat{\sigma}}{\hat{\mu}} \qquad (2)$$

Where $\hat{\sigma}$ is the estimated standard deviation and $\hat{\mu}$ is the estimated mean across the five repeated sampling iterations. These parameters were estimated using the MS Excel command *STDEV* and *AVERAGE*, respectively.

2.5.2 Sampling bias when hooking fish

Fish captured using the seine net were assumed to be an unbiased representation of the entire cage population and their attribute estimates were used as a proxy of the true values. The accuracy of the hook-and-line sampling strategy was then assessed by comparing the profile and parasite burden of fish captured using the hook-and-line with the fish captured with the seine net. Fish profiles were compared using their bodyweight (Kg), fork length (cm), and 'fish class'. Fish classes were defined using the extreme 5th percentile of weight or fork length of the seined fish and sorted into three categories; i) *Low extreme fish* (LE) – less than or equal to the 5th percentile of fork length (FL) and of the 5th percentile of bodyweight (W), ii) *High extreme fish* (HE) – greater than or equal to the 95th percentile of FL and of the 95th percentile of W in the seined fish, and iii) *Normal fish (N)* – the 'normal' range of FL or W. Relative risk was estimated to compare proportions of extreme fish class between the two capture techniques. Parasite burden were conventionally reported using prevalence (proportion of parasitised fish), abundance (average parasite count per fish present), and intensity (average parasite count per infested fish) (Margolis et al., 1982). To account for the fact that ectoparasite carrying capacity is highly host's size dependent, fluke burdens were compared across fish classes using 'fluke density' (fluke count per Kg of bodyweight). When data distributions deviated from normality, a non-parametric Wilcoxon rank-sum test was used to compare two groups' distributions (bodyweight, fork length, parasite counts) and the Kruskal-Wallis equality-of-populations rank test to compare more than two groups' distributions. Significance was determined at a 5% level. When multiple post-hoc comparisons were performed, a Bonferroni correction was applied (5% divided by the number of possible pairwise comparisons) to adjust the level of significance.

3. Results

3.1 Repeatability of the industry protocol

Because the YTK industry protocol uses pooled count, only abundance (average fluke count per fish) of *Z. seriolae* (GF) and *B. seriolae* (SF) can be used in routine to monitor and report fluke burden by the YTK industry. The abundance of each of the replicated industry fluke assessments and their respective repeatability coefficient (CR) and coefficient of variation (CV) are summarized in Table 1. Overall, the study sea cage had a high abundance of GF mainly juveniles (average of 131.4 juvenile and 14.4 adult GF per fish) and mild abundance of SF (average of 5.92 juvenile and 0.48 adult SF per fish). The profile of parasite burden in the study cage was consistent with a wide spread and maturing fluke infestation (or re-infestation) with an abundant burden of juveniles and low but probably increasing number of mature adult flukes. The stage of infestation of GF in this particular cage seemed more advanced than the infestation of SF.

3.2 Sampling bias of hooking fish

3.2.1 Fish profile

Fish sampled using the hook-and-line method had a different fork length (FL) and weight (W) distribution profile, compared to fish captured with the seine net (Fig. 1). The FL distribution in hooked fish appeared mixed visually and was found to be significantly different from seined fish (Wilcoxon rank-sum test *P*-value = 0.016). The W distribution in hooked fish appeared also mixed visually and was significantly different from the seined fish (*P*-value = 0.009). Overall, hook-and-line was significantly more likely (29%) to capture YTK from the extreme fish classes (LE, low extreme or HE, high extreme) compared to the seine (7%) (RR

= 4.14, 95% CI: 1.90–9.01, *P*-value = 0.0001). In detail, 23% of YTK captured with hook-and-line were LE fish compared to 4% with the seine (RR = 5.75, 95% CI: 2.06–16.03, *P*-value = 0.0001), and 6% were HE fish with hook-and-line compared to 3% for the seine (RR = 2.00, 95% CI: 0.51–7.78, *P*-value = 0.3062). The order of hooking (10-20 fish at the time) did not change the bias towards LE fish class (Fisher exact test *P*-value = 0.983).

3.2.2 Parasite burden

Only the initial visual count of SF was used for analysis because the SF count from freshwater bathing was lower and somewhat less reliable (some SF could not be properly dislodged and recovered from the bath's water). According to the seine net sampling (reference method), all YTK in the study cage were infested with juvenile and adult GF (prevalence = 100%), and with juvenile SF but only 24% of the fish were infested with adult SF (Table 2). On average, juvenile flukes outnumbered adult flukes which was consistent with an early stage of fluke colonisation of the study cage. The distributions of counts of juvenile and adult GF and juvenile SF in hooked fish were significantly different than in seined fish (*P*-values < 0.01, Table 2). The main difference was a lower abundance of juvenile GF and a higher abundance of adult GF in hooked fish which was consistent with a more advance stage of GF infestation in hooked fish (i.e. juvenile flukes that matured into adults). The observed difference in juvenile SF counts, although significant, was small (1.82 flukes difference). To account for fish size, burden were compared using 'fluke density' (fluke count per Kg of bodyweight). Fluke density was consistently higher in hooked fish (Table 2). Despite significance for some categories, the observed difference in fluke density between capture techniques were too small to impact the interpretation of the stage of infestation in the study cage and to change the treatment decision.

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Regardless of the capture technique, the fluke counts appeared to be lower in LE fish compared to the other two fish classes (N, HE) (Table 3). This can be explained by the smaller body size of LE fish. Compared to N, LE fish had a significantly higher density of juvenile GF (difference: +30.66 parasites/Kg), adult GF (difference: +6.48 parasites/Kg), juvenile SF (difference: +3.46 parasites/Kg) and adults SF (difference: +0.36 parasite/Kg) (all significant at the Bonferroni adjusted P-values). Compared to HE, LE fish had a significantly higher density of juvenile GF (difference: +99.83 parasites/Kg), adult GF (difference: +6.57 parasites/Kg) and juvenile SF (difference: +3.99 parasites/Kg) (all significant at the Bonferroni adjusted P-values). Density of adults SF were not significantly different between LE and HE fish (difference: +0.40 parasite/Kg). Fluke density of HE fish was consistently lower but did not differ significantly from N fish.

4. Discussion

4.1 Repeatability (precision) of the industry protocol

The current industry fluke monitoring protocol (10 pooled hooked fish) revealed moderate repeatability in the measurement of fluke abundance (relatively large CR and CV) (Table 1). Regardless of the observed variability across assessment iterations, the decision about intervention would not have change in the particular situation of the study cage (i.e. treating). The imperfect precision of the method may be explained by the high biological variability of parasite count within the study cage (e.g. from 42 to 355 juvenile GF per fish) and the relatively small sample size. Individual fluke count from seined fish indicated that the study cage was experiencing a wide spread (100% fluke prevalence except for adult SF) maturing fluke infestation (higher juvenile over adult fluke burden) (Table 2). The dispersion

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of parasite counts reflected different infestation and maturation stages among fish. This is consistent with a lag in time of infestation across fish and a progressive infestation within the cage. More homogenous fluke burden and, therefore, higher repeatability in fluke abundance measurements would be expected when infestation is synchronous. Increasing the sample size (n > 10) is expected to improve repeatability only if the dispersion of parasite count is high. It is difficult to predict if the imperfect repeatability of the monitoring approach could impact treatment decision in different fluke burden scenarios. Further evaluation of the repeatability in YTK cages with different infestation presentations should confirm the impact of parasite count variability on the precision of the sampling approach and the need to adapt the sample size.

4.2 Selection bias of hooking fish

It is generally accepted that hooking fish is highly selective and rarely represents the sampled population and that capturing caged fish tends to select larger dominant and healthier fish (Oidtmann et al., 2013). The hook-and-line method encompasses a variety of approaches and options that can easily affect the selective nature of the technique. Factors of importance include the reach of the line (e.g. use of a rod and/or reel, length and strength of the rod and of the line), type of terminal tackle (size of the hook, use of bait or lure), and skills of the angler (agility and experience). For instance, the size and type of the terminal tackle directly influences the size range of the captured fish (Hetrick and Bromaghin, 2006). The fishing rods used in the study were short (1.5–2 m) carbon fibre stick with no reel and the nylon line was approximately half the length of the rod with a size 8/0 hook without bait or lure (Fig. 2). Only fish swimming at the surface on the immediate edge of the cage could be reached using this setting. The hook-and-line did not favour the capture of larger fish as was hypothesized, but

favoured the capture of the smallest and poor performing YTK in the study cage (LE fish class, Fig. 1). This can be explained in this study by the use of particular hook-and-line setting that may differ from other industry. However, this supports another general believe that poor performing fish tend to gather on the margins of the cage. LE fish had a significantly higher fluke density relative to the rest of the caged population (Table 3), thus their over-representation in the hooked fish resulted into an overestimation of the true fluke density in the sea cage (Table 2). Due to the cross-sectional nature of this survey, it is not possible to differentiate between the LE fish being smaller subsequently to the higher fluke burden, or LE fish being more likely to be re-infested due to lower immunity or increased exposure to fluke when swimming along the net of the cage (reverse causation).

4.3 Parasite control implication

Despite the current industry protocol providing an imprecise, restricted and overestimated measure of the true fluke burden, the interpretation of the infestation stage in the study cage would had limited impact on the proper control of flukes. Currently, treatment trigger points are based on either ten adult GF or ten adult SF per fish, corresponding to the start of fluke eggs release and subsequent spread amplification of fluke infestation in the cage and in the farm. In this instance, the intervention threshold to treat a cage was passed in each of the sampling iterations and, according to the true burden estimate, the farm manager would have correctly decide to treat. Averaging the dislodged flukes of several pooled fish (industry protocol) limits the interpretation of parasite burden to parasite abundance (average parasite count/fish). Individual fish fluke counts would provide important parasite dynamics information about the prevalence (proportion of infected fish) and intensity (average parasite count/infected fish) for management decisions. The homogeneity of the fish infestation in the

cage could trigger very different decisions. For instance, given similar abundances (i.e. low), control strategies may differ widely if most fish in the cage carry very few parasites (high prevalence, and low intensity) compared to if only few fish in the cage carry a lot of parasite (low prevalence, and high intensity). The latter scenario would require an urgent intervention to protect the health and welfare of heavily parasitised fish. In this particular case, the selection bias from hooking would not have led to detrimental delays in the decision to treat, but may have resulted in treatment being provided earlier than necessary. This selection bias did not jeopardise the control strategy adopted by the industry to limit the release of fluke eggs in the farm environment, and supported farm management to relax bathing schedules according to resources and capacities. It should be considered that the magnitude of sampling bias introduced by hooking fish may differ according to the homogeneity of the fish cage, wherehooking fish from heterogenous cages (fish profile and parasite burden varies greatly), may introduce a larger bias than compared to homogeneous cages (fish with very similar in profile and parasite burden). Further sampling evaluation of hooking in cages presenting different variability of fish profile and parasite burden should be conducted.

In general, the current industry protocol to assess fluke burden is likely to suit the aggressive approach of the industry to control GF and SF. Ongoing assessment and review of fluke monitoring programs may help lower the required treatment interventions through optimal timing while maintaining adequate fluke control, thus improving fish welfare and lowering production costs.

4.4 Study Limitations

Limitations to this study include the assumption that the seine net method was a 'true' representative sample of the cage population, the accuracy and comparability of the individual

fluke count, and the restriction to a single study cage. In the absence of a rigorous random sampling process, it is uncertain that the seined fish were not itself a biased representation of the cage population. An alternative would be to conduct the study using a systematic random sampling process at cage grading, transfer or harvest, as described in Oidtmann *et al.* (2013).

Microscopic counting of GF and macroscopic counting of SF encompasses a high degree of subjectivity in the identification and detection of small juvenile flukes, potentially leading to a measurement bias and underrepresentation of this category of fluke. However, in this study, this bias was most likely to be consistent across sampling groups, thus not impacting upon comparisons of sampling strategies. Although fish populations are generally considered relatively large and homogenous (Oidtmann et al., 2013), it was shown the study population was highly variable in size and parasite burden (Figure 1, Table 3). In order to compare the parasite burden between fish of different size, we included the standardisation of fluke count by Kg of bodyweight (density). However, using bodyweight may not provide the best approach to reflect for the carrying capacity of YTK for ectoparasites. Further morphometric research on YTK to identify and validate a proper proxy for body surface area or gill surface area should potentially provide a more robust measure of fluke density.

Lastly, the access to a single cage for this study limits the generalizability of its findings to routine fluke management. As has been shown in other sea cage scenarios parasite abundance seemed clustered with most variation occurring between cages instead of within (Revie et al., 2007). According to the degree of homogeneity of the fish in the cage being monitored, hooking fish will generate different degree of estimation bias in fluke abundance. Evaluating hook-and-line capture across a range of fish cages would provide a better understanding and expectation of the incurred sampling bias.

5. Conclusion

Despite an imperfect precision and accuracy in the current cage-level assessment of fluke burden, understanding the magnitude and direction of the bias allows informed management decision to still be achieved using the current industry protocol. Not by intention this potential bias suited the desired approach of the current management program in this specific cage scenario (i.e. threshold for treatment), as a tendency to overestimate fluke burden safeguards a conservative approach to control GF and SF in commercial YTK sea cage systems. Sampling error when capturing fish from sea cage is difficult to predict and likely variable in nature in different cultured fish species and across a range of parasite burden and fish profile scenarios.

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Table 1. Abundance (average fluke count per fish), mean, median, repeatability coefficient (CR) and coefficient of variation (CV) of juvenile and adult fluke counts from five iterations of the industry assessment (pool of 10 fish per assessment) in a same sea cage of farmed yellowtail kingfish (YTK, *Seriola lalandi*). gill fluke (*Zeuxapta seriolae*) were counted microscopically from a pool of 10 YTK, while skin fluke (*Benedenia seriolae*) were counted visually for each individual YKT.

Iteration	*Zeuxapta seriolae*			*Benedenia seriolae*		
	Juvenile	Adult	Total	Juvenile	Adult	Total
1	104	12.3	116.3	5	0.6	5.6
2	162	16.8	178.8	6.3	0.3	6.6
3	79	11.6	90.6	5.8	0.6	6.4
4	135	15.2	150.2	5.4	0.8	6.2
5	177	16	193	7.1	0.1	7.2
Mean	131.4	14.4	145.8	5.92	0.48	6.40
Median	135	15.2	150.2	5.8	0.6	6.4
CR	114.2	6.5	120.4	2.31	0.78	1.65
CV	30.7%	16.0%	29.2%	13.8%	57.8%	9.1%

Table 2. Comparison of the prevalence (proportion of infested fish), intensity (median parasite count per infested fish) and abundance (average parasite count per fish) of juvenile and adult gill fluke (*Zeuxapta seriolae*) and skin fluke (*Benedenia seriolae*) in a farmedyellowtail kingfish (YTK, *Seriola lalandi*) sea cage population when sampled by either hook-and-line (n = 100) or seine net (n = 100). Difference between hooked and seined fish in prevalence were compared using the Fisher's exact test; while intensity and abundance distribution were compared using Wilcoxon rank-sum test.

Parasite burden	Seine net (reference method)	Hook-and-line	Difference	*P*-value
Gill fluke (*Zeuxapta seriolae*)				
Juvenile				
Prevalence	100.0 %	100.0 %	0.0%	1.000
Intensity (range)	175.5 (43–355)	148 (42–349)	-27.5	0.0088
Abundance	176.0	153.1	-23.1	0.0088
Density (fluke/Kg)	100.0	99.2	-0.8	0.5625
Adult				
Prevalence	99.0 %	100.0 %	1.0%	1.000
Intensity (range)	9 (1–43)	17 (3–35)	8.0	< 0.0001
Abundance	11.3	17.7	6.4	< 0.0001
Density (fluke/Kg)	6.5	11.6	5.1	< 0.0001
Total				
Prevalence	100.0 %	100.0 %	0.0%	1.000
Intensity (range)	190.5 (50–338)	164.5 (45–380)	- 26.0	0.0743
Abundance	187.3	170.9	-16.6	0.0743
Density (fluke/Kg)	106.5	110.8	4.3	0.8079
Skin fluke (*Benedenia seriolae*)				
Juvenile				
Prevalence	99.0 %	100.0 %	1.0%	1.000
Intensity (range)	4 (1–13)	7 (2–13)	3.0	< 0.0001
Abundance	4.84	6.66	1.82	< 0.0001
Density (fluke/Kg)	2.88	4.37	1.49	< 0.0001
Adult				
Prevalence	24.0 %	28.0 %	4.0%	0.6289
Intensity (range)	1 (1–9)	1 (1–7)	0.0	0.8180
Abundance	0.40	0.47	0.07	0.5465
Density (fluke/Kg)	0.22	0.34	0.12	0.4274
Total				
Prevalence	99.0 %	100.0 %	1.0%	1.000
Intensity (range)	5 (1–13)	7 (2–13)	2.0	< 0.0001
Abundance	5.24	7.13	1.89	< 0.0001
Density (fluke/Kg)	3.10	4.71	1.61	< 0.0001

Table 3. Comparison of the prevalence (proportion of infested fish), intensity (median parasite count per infested fish), abundance (average parasite count per fish) and density (average parasite count per fish per Kg of fish weight) of juvenile and adult gill fluke (*Zeuxapta seriolae*) and skin fluke (*Benedenia seriolae*) in low extreme (LE) fish class (FL $\leq$ 44.5 cm and W $\leq$ 1.36 Kg), high extreme (HE) fish class (FL $\geq$ 51.0 cm and W $\geq$ 2.15 Kg), and normal (N) fish class (other than LE or HE) of farmedyellowtail kingfish (*Seriola lalandi,* n = 200). Prevalences among fish classes were compared using the Fisher's exact test; intensity and abundance distributions were compared using Kruskal-Wallis equality-of-populations rank test; and density averages were compared using linear regression. Per row, estimates sharing the same superscript letter were not significantly different from each other (adjusted with Bonferroni correction to account for multiple comparisons).

Parasite burden	'Low Extreme' Fish Class (n = 27)	'Normal' Fish Class (n = 164)	'High Extreme' Fish Class (n = 9)
Zeuxapta seriolae			
Juvenile			
Prevalence	100.0 % [A]	100.0 % [A]	100.0 % [A]
Intensity (range)	136 (42–239) [B]	168 (43–349) [A]	145 (67–284) [A,B]
Abundance	128.7 [B]	170.3 [A]	168 [A,B]
Density (fluke/Kg)	**127.02**	**96.36** [A]	**76.17** [A]
Adult			
Prevalence	100.0 % [A]	99.4 % [A]	100.0 % [A]
Intensity (range)	15 (3–32) [A]	13 (1–43) [A]	17 (6–30) [A]
Abundance	14.4 [A]	14.4 [A]	18 [A]
Density (fluke/Kg)	**14.67**	**8.19** [A]	**8.10** [A]
Total			
Prevalence	100.0 % [A]	100.0 % [A]	100.0 % [A]
Intensity (range)	149 (45–249) [B]	182.5 (50–380) [A]	175 (88–310) [A,B]
Abundance	143.0 [B]	184.6 [A]	186 [A,B]
Density (fluke/Kg)	**141.69**	**104.54** [A]	**84.27** [A]
Benedenia seriolae			
Juvenile			
Prevalence	100.0 % [A]	99.4 % [A]	100.0 % [A]
Intensity (range)	6 (4–13) [A]	6 (1–12) [A]	6 (3–11) [A]
Abundance	6.6 [A]	5.6 [A]	5.9 [A]
Density (fluke/Kg)	**6.64**	**3.18** [A]	**2.65** [A]
Adult			
Prevalence	37.0 % [A]	24.4 % [A]	22.2 % [A]

Intensity (range)	1 (1–6) [A]	1 (1–9) [A]	1 (1–1) [A]
Abundance	0.63 [A]	0.41 [A]	0.22 [A]
Density (fluke/Kg)	**0.60** [B]	**0.24** [A]	**0.10** [A,B]
Total			
Prevalence	100.0 % [A]	99.4% [A]	100.0 % [A]
Intensity (range)	7 (4–13) [A]	6 (1–13) [A]	6 (3–11) [A]
Abundance	7.2 [A]	6.0 [A]	6.1 [A]
Density (fluke/Kg)	**7.24**	**3.42** [A]	**2.75** [A]

Fig. 1. Crossed histogram and scatter plot of farmed yellowtail kingfish (YTK, *Seriola lalandi*) fork length (FL, cm, y-axis) and bodyweight (W, x-axis) sampled from a commercial sea cage using either seine net (A, n = 100) or hook-and-line (B, n = 100). Dashed lines indicate the 5th and 95th percentile of the FL and W of the seined fish (A) used to define the extreme fish classes: low extreme (LE) fish class (FL $\leq$ 44.5 cm and W $\leq$ 1.36 Kg), high extreme (HE) fish class (FL $\geq$ 51.0 cm and W $\geq$ 2.15 Kg).
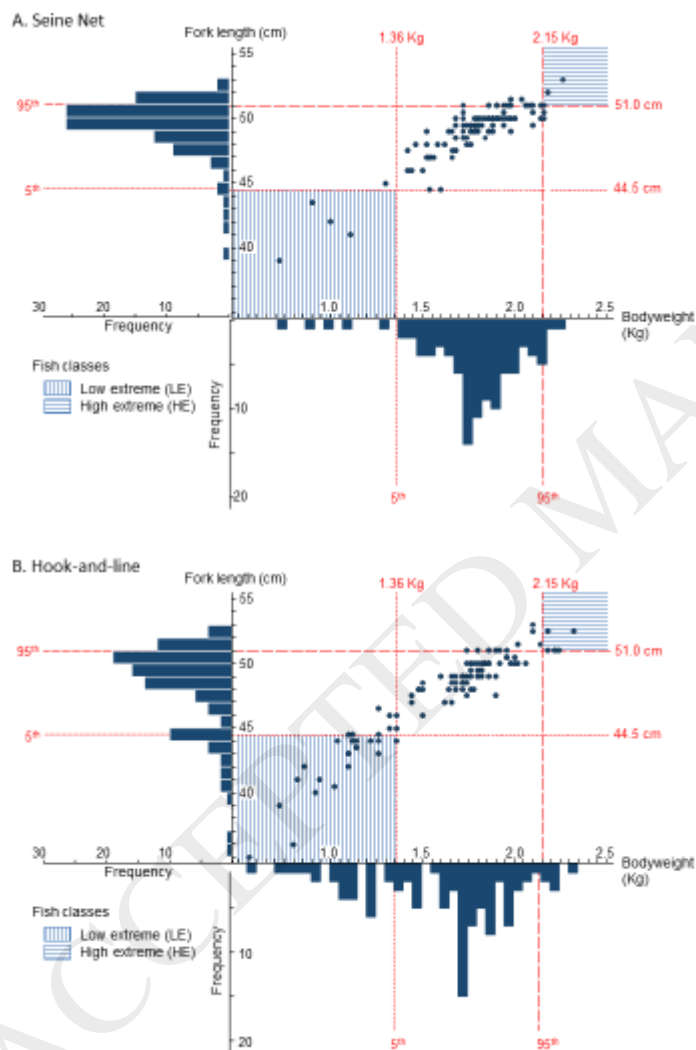
Fig. 2. Industry sampling method using to the hook-and-line to conveniently capture farmed yellowtail kingfish (YTK, *Seriola lalandi)* from the edge of a 40 metre diameter circle sea cage in Boston Bay, Port Lincoln, South Australia.