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Methods for sea lice surveillance on marine finfish farms

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National foreword

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Methods for sea lice surveillance on marine finfish farms

Méthodes de surveillance des poux de mer dans les exploitations de pisciculture marine

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Foreword

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The committee responsible for this document is ISO/TC 234, *Fisheries and aquaculture*.

Introduction

The term “sea lice” refers to several species of naturally occurring marine copepods that parasitize fish. They attach themselves to the skin, fins, and gills of wild and farmed fish, and feed on host mucus and skin. If an infestation is severe, it can negatively impact the health of affected fish.

The abundance of sea lice can be amplified on marine finfish farms and a concern is that farms can then act as a reservoir, releasing lice back into the broader marine environment. Farms could thus act as a source of infestation for wild fish, particularly salmonids. While it is possible to control lice levels on farms through integrated pest management approaches and the use of therapeutants, the same intervention cannot easily be applied to wild fish populations. For this reason, many jurisdictions place requirements on farmed salmon producers to carefully monitor lice levels on farms and to take appropriate actions to reduce on-farm lice populations where lice have been identified as a concern.

Where sea lice are identified as a concern to be managed, counts may be used in a number of ways. Over the past decade, it has become increasingly common for regulators to establish upper limits to abundance of lice on farms with the intent of minimizing potential impacts to wild fish populations. Farming companies can also monitor lice abundance to maintain appropriate fish welfare conditions. In addition, the development of reduced sensitivity to in-feed medication (following similar trends seen for bath treatments in the 1990s), is a concern to both regulators and producers. Clear and standardized sea lice counts are necessary for the early detection of any such trends. Both the aquaculture industry and pharmaceutical suppliers of ecto-parasiticides would then be in a position to make better and earlier determination of situations in which treatments were beginning to lose effectiveness, and thus, to initiate appropriate mitigation strategies.

Over the past two decades, a range of counting methods have been developed across countries, and sometimes within countries, such that it is often difficult to know how to interpret the sea lice levels reported from farm sites. The goals of this International Standard are to ensure that the sea lice counts carried out on marine finfish farms are accurate and fit for purpose and to establish a method for sea lice on-farm surveillance that can be carried out in any farming area, affording accurate, consistent estimates of lice. A standardized methodology will yield results that can better be compared across jurisdictions and geographic regions, supporting the development and implementation of effective lice management approaches and increasing public confidence that effective control measures are being implemented.

This International Standard has been developed in consideration of the

— intended use of the results of sampling,

— practical and economic constraints of sampling,

— species of lice of concern in an area,

— cage and site configurations,

— seasonal or environmental conditions, and

— potential impacts on fish health and welfare.

Methods for sea lice surveillance on marine finfish farms

1 Scope

This International Standard specifies both a method for sea lice counts on marine finfish farms and a method for sea lice surveillance that can be carried out in any farming area to provide consistent estimates of sea lice infestation.

It specifies the best practices associated with monitoring sea lice levels on marine finfish farms for various purposes including the assessment of abundance, prevalence, and treatment efficacy. This will include identifying minimum requirements for specific monitoring program elements (e.g. number of fish and cages to be sampled, frequency of sampling, the level of detail recorded, etc.). The standard will apply to all marine finfish farms which experience infestation with any of the range of "sea lice" (copepodid) parasites.

2 Normative references

There are no normative references in this International Standard.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
sea lice
copepods of one of a number of lice species

Note 1 to entry: The most commonly occurring sea lice, depending on location, being the various salmon lice *Lepeophtheirus salmonis* Krøyer, *Caligus elongatus*, *Caligus clemensi*, *Caligus rogercresseyi*, etc. and the cod louse *Caligus curtus*.

3.2
sea lice stages
sea lice metamorphose to different life stages

Note 1 to entry: Sea lice stages includes the nauplii (free-swimming stage) through the copepodid stage (infectious stage) to various stages of chalimus growth (attached stages) where they are attached to a single point on the fish host. They then develop to pre-adult (for some lice species) and finally, adult stages, at which point they are able to move around on the fish host (motile stages).

3.3
finfish
fish of the class Osteichthyes

3.4
facility
collective structures used for the purposes of finfish aquaculture; including the enclosures (net pens), walkways, barges, floats plus associated lines and anchors

3.5
enclosures
containment structures, including net pens, cages, or similar structures used to contain finfish for the purposes of aquaculture

3.6
treatment
measure applied to remove sea lice

EXAMPLE Treatment such as in-feed drug products, topical pesticides, or other methods that remove lice, including mechanical removal.

3.7
abundance
mean number of lice across all of the fish that are examined at any particular sampling point

3.8
prevalence
proportion of fish on which at least one louse was observed from all of those examined during any particular sampling event

3.9
intensity
mean number of lice across only those fish on which lice have been observed during the examination at any particular sampling point

3.10
accuracy
quantitative measure of the degree of conformity with an accepted reference value

[SOURCE: ISO 6707-1:2004, definition 9.1.8]

3.11
precision
quantitative measure of the degree of agreement between individual measurements of the same property

[SOURCE: ISO 6707-1:2004, definition 9.1.9]

3.12
sea lice count
event involving a number of fish from a range of cages on a marine finfish farm on a specific date which enumerates the presence of different sea lice stages on these fish

3.13
sea lice surveillance
process by which sea lice levels are recorded and assessed over time; the protocol to organize and assess a series of sea lice counts

3.14
clustering
degree to which parasites tend to aggregate within units (in this case cages)

Note 1 to entry: Can be formally measured by the intraclass correlation coefficient (ICC) which describes how closely communities in the same unit tend to resemble each other.

4 Sampling design

4.1 Purpose, precision, and accuracy

The most important consideration in determining sampling design is the purpose for which the monitoring is being carried out. The key issue in deciding on a sampling strategy is specifying the level of precision or accuracy that is required as an outcome from the sampling process.

NOTE 1 The purpose of monitoring could be to fulfil regulatory requirements, in which case, it would be up to the relevant authority to decide on the design details, or it could be to monitor the effectiveness of the treatments for sea lice, in which case, the outcome could be of importance to both the authorities and producers of fish. The design and frequencies of the monitoring will therefore vary.

NOTE 2 When reporting mean sea lice levels for comparison against treatment triggers, or simply to visualize trends over time, it can be that ±20 % is adequate, while estimating efficacy of a treatment intervention can require a more tightly defined precision.

There are also a number of biological factors that will affect the accuracy of estimates. In particular, the level of clustering which exists in a given situation will impact on the importance of selecting a representative number of cages from within a farm.

NOTE 3 Clustering refers to the relationship of within-cage to between-cage variation and is known to be important in most ecological monitoring situations.

4.2 Specification of measure to be used (abundance, prevalence, and median intensity)

4.2.1 General

It is not only precision and accuracy that are associated with purpose; the actual metric that is used to express the level of sea lice load on fish may change in differing contexts and under varying conditions (see also Annex B). Abundance is the most commonly used metric when sampling sea lice. However, when sea lice levels are very low, as is assumed to be the case when trigger levels of, for example, 0,1 adult females per fish are in place, it is unlikely that abundance will be the best metric.

NOTE 1 General advice from the quantitative parasitology literature is that prevalence and median intensity may be better metrics to use than mean abundance in this circumstance.

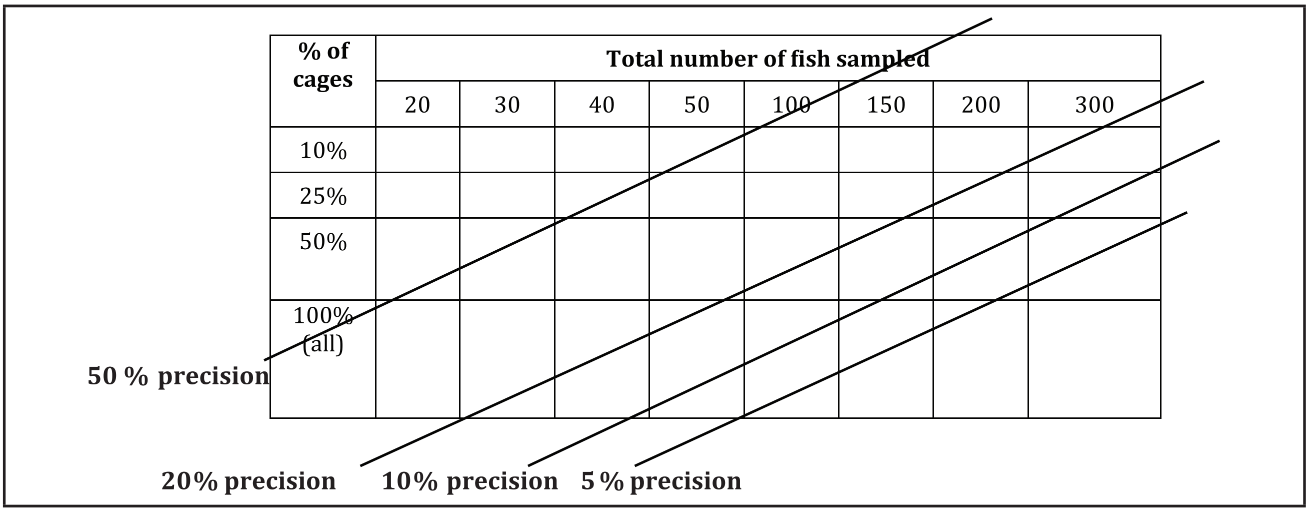
NOTE 2 Regulatory thresholds are usually based on average sea lice figures, or in parasitological terminology, “abundance”; while estimates of intensity and prevalence are typically only used in epidemiological studies.

4.2.2 Abundance

Having taken note of the considerations above, a relevant level of accuracy should be selected. The cells contained in Table 1 indicate the estimated levels of accuracy that can be expected to be obtained under different configurations of cage coverage and total number of fish in the sample. The accuracy increases as the total number of fish sampled is increased (i.e. from left to right) and under normal conditions, where clustering is present, the same is true as a larger proportion of the cages is included (i.e. moving from top to bottom in the table). The diagonal lines in Table 1 indicate sampling strategies of equivalent accuracy.

NOTE The actual slope of the diagonal lines in Table 1 is dependent on the degree of clustering present in a given situation. The metric used to define this is the Intra-class Clustering Coefficient or ICC, which in the example shown, based on empirical data, has been set to a value of 0,35. As the ICC approaches a value of zero, i.e. no clustering, the lines of accuracy-equivalence would tend towards the vertical, indicating that accuracy is based only on total number of fish included in the sample.

Table 1 — Impact of number of fish and % of cages sampled on precision estimation



4.2.3 Prevalence and median intensity

In situations of low abundance (e.g. less than 0,2 individuals per fish), an alternative metric should be used to report sea lice infestation. This may be in addition to or in place of abundance. In such situations, the prevalence will also be low, in the region of 10 % to 15 % of fish having parasites. Similar general issues as those outlined in 4.1 will hold when determining accuracy. However, for prevalence, the maximum level of resolution in any estimate will be limited to $1/N$ (where N is the number of fish per cage, or per site, depending on the level at which prevalence is being estimated). Intensity measures consider only fish which have sea lice present on them, and thus, in such situations, taken with prevalence, can provide a more useful summary of infestation than abundance. In addition, by using a median value, they can also avoid the problems of bias introduced by a single ‘outlier’ in the circumstance where only a few fish are likely to be infected.

4.2.4 Minimum requirements for on-farm monitoring of sea lice levels and effect of treatment

Having taken note of the considerations in 4.1 and 4.2, the operator should be able to identify an appropriate approach for the on-farm monitoring of sea lice levels and to assess the effect of treatments. However, for the purpose of this International Standard, minimum requirements for such monitoring are set. The monitoring of sea lice levels is in Table 2 and the monitoring of the effect of treatment for sea lice is in Table 3. Table 2 and Table 3 both sets out the minimum requirements in terms of proportion cages and numbers of fish sampled for various scenarios.

Table 2 — Minimum sampling coverage, number of fish and percentage of cages when monitoring the sea lice levels

Number of cages present on finfish farm	% of cages sampled	Minimum number of fish to be sampled per cage
3 or less	100 %	10
4 to 12	50 %	10
More than 12	33 %	10

Table 3 — Minimum sampling coverage, number of fish and percentage of cages when monitoring the effect of treatment for sea lice

Number of cages present on finfish farm	% of treated cages sampled	Minimum number of fish to be sampled per cage before and after treatment
3 or less	100 %	20
4 to 12	100 %	20

Table 3 *(continued)*

Number of cages present on finfish farm	% of treated cages sampled	Minimum number of fish to be sampled per cage before and after treatment
More than 12	100 %	20

5 Information requirements for sampling events

5.1 General

Adequate sampling shall include several key elements. The contextual elements that shall be recorded are listed in Table 4, while the information specific to the sea lice present on the sampled fish is noted in Table 5.

5.2 Contextual elements at each sampling event

Each time fish are sampled to assess sea lice infestation, a number of contextual elements, which are important to the appropriate interpretation of the observation (see Annex A), shall be recorded as listed in Table 4.

Table 4 — Core contextual elements for sea lice sampling

Level of detail	Type of element		
	Event	Production	Environmental
Facility	Date of sampling Unique identifier for the facility or site	Total number of fish being held on the facility Total number of enclosures holding fish on the facility at the time of sampling Year class of fish stocked at the facility	Seawater temperature, normally at one or more standardized depths (e.g. surface or 3 m) with depth specified Salinity normally at one or more standardized depths (e.g. surface or 3 m) with depth specified
Enclosure	Unique identifier for the enclosure (pen, cage) Method used to catch fish from enclosure Date and product used in most recent use of sea lice treatment	Enclosure volume Estimated average weight of fish in the enclosure Estimated number of fish in the enclosure	
Fish		Observations of lice-related damage to the fish Poor performing fish	

5.3 Sea lice elements at each sampling event

Once the major lice species of concern has been identified (in many regions, this will be *Lepeophtheirus salmonis*), recorded observations of all sea lice on fish shall include annotation of the lice species as well as enumeration of specific life stages. The sea lice life stages of interest are the chalimus (also referred to as "attached") stages and the motile stages (which include adult male and adult female, and for some species, pre-adult life stages).

Some rationale as to the importance of recording details of these various stages can be found in Annex A. Sea lice numbers on the cultivated fish are to be counted, by visual observation with the naked eye, and recorded as indicated in Table 5.

Table 5 — Sea lice elements for sampling

Stage	Major (dominant) sea lice species	Minor sea lice species	None recorded
Female	Motile stage of adult female	Total individuals in any motile stage	A “no sampling” report[c]
Motiles	Motile stage of adult male		
Pre-adult[a]	Motile stage of pre-adult lice		
Chalimus[b]	All chalimus or attached stages		

[a] In some cases, it may be acceptable to simply record all motile stages, other than adult females, as a single variable (i.e. adult males plus pre-adult stages). However, the recommendation of this International Standard would be to record pre-adult, adult female and other motiles separately. At a minimum, the number of adult females, as well as the number of all motile lice shall be recorded regardless of what other differentiation is recorded.

[b] Visual observation with the naked eye is limited. If there is a need to speciate the chalimus stages, a microscope is needed.

[c] If no monitoring was undertaken due to exclusion (e.g., water temperature or algal bloom precluded sampling), then this should also be recorded.

5.3.1 Sea lice dislodged during handling

Sea lice that may have been dislodged through the handling of fish during sampling are to be accounted for by

a) keeping fish in containers and using a sieve for count of each dislodged parasite, and

b) visually inspecting the container, with methodology varied according to the targeted sea lice indicator:

   1) if the sampling design is targeting the calculation of abundance (i.e. mean number of sea lice per fish), then the number of sea lice in the sampling container can be totalled at the end of a given cage sampling event and pro-rated across all the fish sampled, or

   2) if the sampling design is targeting the calculation of prevalence or intensity of lice on fish, then inspection for lice in the sampling container shall be to be carried out as each fish is inspected. (If in a given situation this is not possible, then the user should be aware that intensity/prevalence measures cannot be adequately estimated.)

5.4 Structured format for reporting

The reporting of lice sampling events (both contextual and sea lice elements) shall be carried out in a structured fashion. Only by using a reporting method that is well-defined and consistent over time can useful temporal trends be created and interpreted. An example of the type of data sheet that should be used to report sea lice sampling events is provided in Annex B. In some cases, these types of data may be entered directly into mobile digital devices. In others, the data will initially be recorded on physical media. In the latter case, it is important that the data be transferred to a digital format if long-term storage and trend analysis is to be carried out.

6 Monitoring programme elements

6.1 General

Any monitoring programme shall specify the frequency at which sampling is to take place as well as the method for selecting and handling fish.

6.2 Selection of fish and handling

Fish should be handled in a manner that minimizes stress, damage to fish, loss of lice, and meets applicable fish welfare requirements including the appropriate use of anesthetic. Anaesthetizing fish before counting lice decreases handling related stress on the fish and reduces the number of lice dislodged during sampling.

A representative sample of fish, as determined by the sampling design (see Clause 4), should be collected from the enclosure by crowding the fish into an accessible area and then removing the fish to be sampled using a dip net. Selected fish should be placed into an anesthetic bath and examined one at a time.

6.3 Frequency for regular monitoring

When establishing frequency of sampling, a range of factors affecting sea lice population dynamics should be considered. These would include elements such as the following:

— sea water temperature;

— salinity;

— migration of wild fish;

— oceanographic conditions;

— treatment;

— presence of other hosts and pathogenesis;

— general or specific legal requirements.

A guideline for typical sampling frequencies is given in Table 6. While these would be the frequencies normally expected, some mitigating factors (such as those listed above) can lead to an increase or decrease in the regularity of monitoring; these are outlined in Table 7.

Table 6 — Frequency of sampling of sea lice

Surface water temperature	Frequency
<4 ºC	*No counting* — sea lice development is slow and sampling may have negative impacts on fish welfare
4 ºC to 10 ºC	Every 14 d
>10 ºC	Every 7 d

Table 7 — Factors that can affect the frequency of sampling of sea lice

Consideration	Increase frequency	Decrease frequency
Presence of susceptible wild species at a vulnerable life stage in the vicinity of the farm	***	
Exceedance of some lice threshold has occurred	***	
To comply with some local cooperative or voluntary agreement	***	
Fish at the facility are broodstock		***
Fish are currently undergoing treatment		***
Local salinity is very low (<15 ppt)		***
Adverse environmental conditions (low air temperature, high winds, low dissolved oxygen, etc.)		***
Advice from a fish health professional (e.g. during a disease outbreak)		***

6.4 Frequency for assessment of treatment efficacy

The presence of sea lice should be assessed before and after treatment with therapeutants to adequately estimate the effectiveness of any intervention. This may require slightly different (more regular or purposeful) sampling frequency to that outlined in 6.3.

In particular, it is recommended that any sea lice reducing treatment should have a count event carried out within one week of the treatment date, and that, at sea water temperatures greater than 13 °C, this be reduced to no more than 3 d prior to the treatment event. In addition, even if the water temperature is below 10 °C, it is suggested that counts be carried out in each of the first two 7 d periods following the termination of the treatment.

These guidelines relate specifically to the context of making use of reactive chemotherapeutic bath treatments. Other treatment options exist which tend to act over a longer time period. For example, in-feed treatments such as emamectin benzoate or teflubenzuron which will be present in the flesh of the fish and act on parasite over longer periods. In additionnewer non-medicated alternatives for controlling sea lice in a more proactive manner will tend to have effects that are seen over a longer time period. While it is equally important to assess the efficacy of these types of treatment, this can largely be achieved through the more regular sea lice monitoring outlined elsewhere in this International Standard.

7 Training requirements

At a minimum, personnel carrying out sampling programmes should be trained to perform the basic tasks of

— collecting contextual information, such as environmental measurements, as per 5.2,

— identifying the appropriate species and life stages of sea lice as per 5.3, and

— proper fish handling techniques including the use of anaesthetics.

Sampling skills should be assessed and verified by a fish health professional annually.

In addition for the long term effectiveness and value of such programmes, personnel involved in sea lice surveillance should also be trained to understand the importance of, and assist in

— drafting sampling programmes,

— verifying sampling programmes, and

— revising sampling programmes.

Annex A
(informative)

Rationale for collecting site and cage-level data

A.1 The need for "contextual" elements in the data collection process (see 5.2)

Contextual elements support analysis as well as interpretation of sampling results. These elements include environmental factors, production information and data related to the sampling event.

Environmental factors such as salinity and water temperature affect the reproductive biology of lice species and therefore can influence the local abundance of sea lice at the time of sampling. Salinity also affects the ability of lice to attach to or infect host fish and is a factor that can be considered when determining the need for use of anti-louse therapeutants or when assessing efficacy of treatments post-application.

Production information is also necessary for statistical analyses of the sea lice data collected. It helps establish the host population abundance, the relationship between the size of sample collected and the population being sampled, and estimates of the size of the overall sea lice population in a given setting. The general condition, age/size, and time at sea of the host population influence the susceptibility of the salmon to infection and are generally considered when interpreting data.

The method by which fish are caught from within a pen/cage should also be noted. While no truly 'random' method exists, knowing how the fish were caught (e.g. feed-and-dip versus seine net with sub-sampling) can be helpful at time of interpretation.

A.2 The need for lice stage details in the data collection process (see 5.3)

The chalimus stage is of interest as this stage actively feeds in the restricted area around its attachment and can have a negative effect on the health of the host fish. In addition, in more proactive control strategies, the expectation is that the development to motile stages can be largely avoided, and thus, chalimus enumeration is critical to effective monitoring. With a proactive strategy, the chalimus stages, to a greater extent, would be of value for predicting changes in the sea lice levels at a farm. It should, however, be noted that it is difficult to differentiate between species at this life stage with the naked eye so all individual chalimus, regardless of actual species, should be counted and recorded as the major species of concern. While this may result in an over-estimation of this stage of the major sea lice species present, it does not require the use of a microscope to carry out the count.

Motile lice are counted as this information can be helpful in predicting changes in sea lice population at a farm. In addition, some regions have regulatory or best-practice guidelines which include a maximum permitted number of all motile sea lice.

The number of adult female sea lice should also be counted and recorded as a separate stage. This stage is arguably the most important for estimation of emerging population dynamics as the females (and her eggs) are the key determinant of new infestations. In some regions, it is stipulated that "gravid females" (i.e. those adult female with eggs) should be recorded separately. However, this is not recommended as some females without egg strings can develop these in a day or two, while others can have just recently shed theirs. Thus, it is recommended that total adult female numbers are recorded, rather than, or in addition to, those which have egg strings ("gravid").

Annex B
(informative)

Typical measures used to summarize sea lice data and their variability

B.1 Example to illustrate typical measures in sea lice data

There are a number of standard terms that will typically be found in descriptions of the level of parasites on fish, each of which helps illustrate different aspects of any infestation. The definition of some of the terms makes reference to the examples in Figure B.1.

	Number examined	chalimus	motiles	adult females
Cage 1	20	18	54	27
Cage 2	15	10	78	46

		Abundance	Intensity [range]	Prevalence [95 % CI]
Cage 1	chalimus	0,9	1,6 [1 - 4]	55 % [33 - 77]
	motiles	2,7	3,2 [1 - 11]	85 % [69 - 100]
	adult females	1,4	2,1 [1 - 7]	65 % [44 - 86]
Cage 2	chalimus	0,7	1,4 [1 - 3]	47 % [21 - 72]
	motiles	5,2	5,2 [2 - 23]	100 %
	adult females	3,1	3,8 [1 - 9]	80 % [60 - 100]

Figure B.1 — Examples of sample results

B.2 Abundance

This measure simply describes the mean number of lice across all of the fish which were sampled, in this sense, it is the closest to what we colloquially think of when we say, “the average”. In the example above in Figure B.1, for Cage 1 we found a total of 18 chalimus on 20 fish observed, and thus, the abundance is simply 18 divided by 20, or 0,9.

B.3 Intensity

One of the problems with a traditional “average” is that it is of limited value when there are many fish on which there are no lice. In our current example, there are no cage/stage combinations with many lice-free fish. However, in Cage 2 in Figure B.1, only 7 of the 15 fish have at least one chalimus present with a total of 10 chalimus lice over 15 fish. The “intensity” measure also provides a mean number of lice, but only on those fish that are infested. Thus, while the abundance of chalimus in Cage 2 is 0,7, the intensity is just over twice that value (i.e. 1,4 chalimus found on each infected fish). It is also usual to report the range, the larger number in which indicates the maximum number of lice found on any one fish, in the case, we have been looking this is 3 chalimi, while for motile lice in Cage 2, the number rises to 23. (Of course, if every fish was found to be infested then the intensity and abundance are identical, as is seen for the case of motile lice in Cage 2.)

B.4 Prevalence

In cases where the overall abundance is very low and many fish have no infestation, it is often informative to report the “prevalence” which indicates the proportion of fish on which there was at least one louse present. Looking at the case of chalimus in Cage 1 in Figure B1, of the 20 fish assessed there were 11 on which at least one chalimus was found. Dividing 11 by 20 gives us a value of 0,55, which we express as 55 % prevalence.

B.5 95 % Confidence Interval (CI)

Each of the three measures noted above are referred to as “point estimates” for the values estimated to be representative for the population of fish from which these samples were taken. There will of course be variability associated with these values and it is often useful to summarize the range of possible values that might be likely to arise. There are a number of ways to express this variability, but all of these (apart from the simple “range” shown in the case of intensity) are reasonably complex from the point of view of statistical interpretation. However, it is also useful to show the “95 % CI” associated with prevalence values, e.g. in the case of the 55 % result for chalimus in Cage 1, expressed as [33 - 77]. This essentially indicates that the “true” percentage of fish that are infested with chalimus is highly likely to lie somewhere in the range between 33 % and 77 %. In general, the smaller the confidence interval, the more likely it is that the point estimate, in this case, for prevalence of lice, is close to the true value. [For those who have an interest in such things, the technically correct interpretation of a 95 % CI is that, if we were to sample from the Cage 1 fish population 20 times, then, in 19 of these cases, the true value would lie within the specified confidence interval.

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