

Bias in estimates of electrofishing capture probability of juvenile Atlantic salmon

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Abstract

We evaluated the effect of the total number of passes used, and the application of block nets, on multi-pass electrofishing removal sampling for estimating juvenile Atlantic salmon (*Salmo salar* L.) abundance and body size distribution. Sites within selected salmon-bearing Norwegian rivers were enclosed by block nets and electrofished for multiple passes (range: 7–13), and capture probabilities and abundances were estimated using the Carle and Strub removal method. We examined for different body size classes: (1) bias in the estimated capture probability and abundance associated with the number of passes used; (2) the potential for bias to be minimized by the use of block nets; and (3) electrofishing-induced mortality. We found that the capture probability estimate was strongly dependent upon the number of passes used, tending to decline with successive pass, with the effect depending on size class. Thus, estimates made using the traditional three-pass approach would result in underestimates of abundance, and biased estimates of size distribution. Smaller juveniles were both more likely to impinge on the block nets and more likely to experience mortality than larger juveniles. Mortality was greatest when water temperature was high (> 18 °C). Our findings indicate that quantitative electrofishing for small juveniles may be unreliable, and that electrofishing at high temperatures should be avoided due to potential high mortality.

Keywords: Atlantic salmon, electrofishing, multi-pass removal, net capture, mortality

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1. Introduction

Electrofishing with portable gear is a standard method for sampling fishes in freshwater (Anonymous, 2003; Vehanen et al., 2010; Argillier et al., 2013), and is the most commonly used method for sampling juvenile salmonids in streams and moderately sized rivers (Bohlin et al., 1989; Korman et al., 2009). The main reason for the widespread use of electrofishing is that it represents a simple, inexpensive and cost-efficient way to catch riverine fishes.

The objectives of electrofishing surveys range from simply determining the prevalence of fishes or characterizing fish species assemblages to estimating abundances by size- or age-group. However, electrofishing may produce biased estimates of these population characteristics because some fish may avoid capture, particularly if only a single-pass is used (Arnason et al., 2005; Bateman et al., 2005). For example, electrofishing capture probability has been observed to increase with increasing body size, both in salmonids (Peterson et al., 2004; Korman et al., 2009; Saunders et al., 2011) and in other fishes (Dauwalter and Fisher, 2007; Hense et al., 2010) so there is potential to over-sample large individuals and produce unreliable estimates of the population body size distribution. A multi-pass removal approach, in which the change in numbers captured on successive electrofishing passes provides estimates of capture probability, may increase the accuracy of abundance estimates (e.g., Zippin, 1958; Carle and Strub, 1978). However, such an approach relies upon several assumptions. Firstly, it is assumed that the probability of capture is constant over successive passes for all fish. Secondly, it is assumed that sampling is conducted on a closed population – i.e. no fish can leave or enter the fished site during sampling. These two assumptions are often violated.

Capture probability has often been observed to decline with successive passes (Borgström and Skaala, 1993), which may result in biased estimates. For example, a simulation study by van Poorten et al. (2017) found that no single removal method performed robustly under conditions of non-constant capture probability, generally causing an underestimate of abundance due to vulnerable fish being captured earlier. Even when assumptions are not violated, removal estimates are only reliable if sufficient numbers of individual fish are present within the fished area – Riley and Fausch (1992) for example estimated that a minimum sample size of 30 individuals within the site was required. A large proportion of the population must be captured to obtain a precise estimate of the population: for example, Zippin (1958) estimated that for a population of 200 individuals 75% would have to be captured to achieve a coefficient of variation of 10% for the abundance estimate.

Juvenile fish are motile so the assumption of a closed population is often violated due to immigration or emigration, resulting in biased estimates. Additional emigration may be initiated due to a flight response of the fish to the disturbance involved in electrofishing (Young and Schmetterling, 2012). Block nets may be positioned around the electrofished area to ensure a closed population (e.g., Peterson et al., 2005; Bertrand et al., 2006), although installation of these is labor intensive.

Electrofishing may be harmful to fish, resulting in injury or mortality through hemorrhage or spinal injury (Snyder, 2003). A wide range of factors has been associated with this including electric current type, voltage, species and body size (Dolan and Miranda, 2004; Clément and Cunjak, 2010). Registration of injury and mortality rates is necessary if the intention is to improve the electrofishing program to minimize adverse effects on the fish. An additional advantage of using block nets is that they aid in counting electrofishing-induced mortality and injury. Undetected dead or injured fish may be entrained by the river flow to later be impinged on the downstream net where they can be counted after each pass.

We evaluate the potential sources of bias when using multi-pass electrofishing for estimating population abundance and body size distribution of juvenile Atlantic salmon (*Salmo salar* L.). In particular, we examine for different size groups: (1) the dependency of

abundance estimates on the number of passes used; (2) the dependency of abundance estimates on the use of block nets; and (3) electrofishing-induced mortality.

2. Material and methods

2.1 Electrofishing surveys

Five salmon-bearing rivers situated in central Norway (the rivers Homla, Ingdalselva, Levangerelva, Toåa and Vindøla; Fig. 1) were selected for electrofishing. These rivers have sympatric populations of Atlantic salmon and brown trout (*Salmo trutta* L.), but the fish communities are dominated by Atlantic salmon. Atlantic salmon within these rivers mainly smoltify in the spring at age 2-5 years, and the juvenile populations in the summer and autumn consist of individuals aged from age 0+ (year of hatching) to 4+ (the fourth year after hatching).

Electrofishing was conducted during daytime within sites that were enclosed with block nets on a total of ten occasions from August to November (2010-2015). Three of the five rivers were surveyed on more than one occasion (Table 1). When rivers were surveyed on more than one occasion, the same site was used (with the exception that the site for Homla in November 2010 was different to the other years due to operational constraints). Criteria for selecting sites were: (1) water depths that were wadeable, allowing back-pack electrofishing over the entire area; (2) channel widths and depths that were suitable for block nets to span the entire channel; (3) water conductivity that was both suitable for the use of the electrofishing gear, and typical of Norwegian rivers; and (4) a relatively similar hydromorphology among sites (with regard to water depth, current speed and riverbed substrate) to minimize the effect of differences in site-specific hydromorphology on electrofishing estimates.

The channel downstream of the electrofishing site was blocked by a fine mesh net (30 m in length, 2 m in depth, with a 5 mm mesh size) before the application of the electrofishing gear to prevent fish escape during electrofishing. The upper part (float line) of the block net was fixed above the surface of the water using sticks and the lower part of the block net was held down with large stones to ensure that the entire water column was encompassed. An additional block net was installed upstream of the site after the first electrofishing pass. An upstream block net was only installed on completion of the first round of electrofishing to ensure that a sufficient sample size had been obtained to justify continuation of the multi-pass survey: installation after this pass allowed the decline in numbers captured with successive passes to be assessed. Electrofishing was done using a TERIK FA-50 model (Terik Technology AS, www.terik.no), a Pulse Direct Current (PDC) generator model which adjusts the voltage applied to the water conductivity so as to minimize the conductivity-induced bias, while maintaining a voltage level low enough to minimize damage to the fish. Voltage varied between 700 and 1050 V, depending on the water conductivity of the site under investigation.

Electrofishing was carried out using the standard method applied in Norway of two field researchers wading upstream through the river in a zig-zag path, one of whom operated the electrofishing gear while the other assisted and took care of captured juveniles. In addition, two people continuously checked the lower block net to collect and retain impinged juveniles. After each pass, all captured juveniles were registered and classified with regard to species and status (alive or dead) and their lengths were measured. From 2013 onwards, the position of capture (whether at the electrofishing gear or in the block net) was recorded to assess the influence of block nets on the estimates of capture probability and abundance. Captured juveniles were kept in containers holding river water and were returned to the river after the electrofishing survey was completed. Repeated electrofishing passes were carried out, with the time from the start of one pass to that of the next pass being at least 30 minutes. Electrofishing was conducted for a larger number of passes than the traditional three-pass

electrofishing approach (7-13 passes, dependent on survey; Table 1). In eight surveys, numbers of Atlantic salmon captured in the final pass were less than 2.2% of total salmon capture in all passes; in two surveys, numbers captured in the final pass were ~8-9%.

After the completion of electrofishing in each site in September 2010, the site's area (between the block nets) and hydromorphological characteristics were measured. Water depth was measured on cross-channel transects separated by 3-5 m. At the same measuring points, the bottom substrate within an iron frame (0.25 m²) was classified and the number of potential hiding places for juveniles was calculated according to the method of Finstad et al. (2007). Water depths were shallow, with mean depths ranging from 10 to 40 cm (see Fig. 1 for surveys in 2010). All sites were dominated by pebble and cobble substrata.

2.2 Analyses

Captured juveniles showed multi-modal length distributions, largely corresponding to different age-classes (Online Supplementary Fig. 1). To enable assessment of the effect of fish size on electrofishing estimates, captured juveniles were classified into three size groups: small juveniles < 60 mm total length that mainly corresponds to young-of-the-year (fish hatched that year), medium juveniles 60 - 95 mm total length mainly consisting of yearlings and older parr, and large juveniles (> 95 mm) mainly correspond to the presmolt group (Elson, 1957) likely to smoltify and leave the river in the following spring. Size-at-age differed between rivers with larger specimens in the lowland Homla, Ingdalselva and Levangerelva rivers, than in the higher-gradient Toåa and Vindøla rivers.

When estimating size-specific capture probability and abundance, we used the Carle and Strub removal method (Carle and Strub, 1978) available in the R-package, FSA (Ogle, 2015). This method was chosen because it typically provides the most reliable estimates (Coxw, 1983). However, estimates from this method were similar to those from the Zippin (Zippin, 1958), Moran (Moran, 1951) and Schnute (Schnute, 1983) removal methods (Online Supplementary Fig. 2), suggesting that for the data used in this study, the specific removal method will have had little effect. Estimates from these methods are unbiased only when the assumptions of constant capture probability and a closed population are met, so variances estimated by these methods are not valid if these assumptions are violated.

To examine whether differences among survey conditions could have influenced capture probabilities and therefore affected our examination of biases, we examined the relationship between capture probability (estimated from all passes, with the number captured on each pass being the sum of those captured at the electrofishing gear and those captured at the block nets, whether alive or dead) and total salmonid density (total capture of Atlantic salmon and brown trout individuals m⁻²) and water temperature using Pearson's *r*. A power analysis was then done (using R-function `pwr.r.test{pwr}`) to determine if the sample size was large enough for us to be confident that we could correctly accept the null hypothesis, based on the observed correlation. We then examined the fish size-specific effect of: (1) the dependency of abundance estimates on the number of passes used; (2) the dependency of abundance estimates on the use of block nets; and (3) electrofishing-induced mortality.

2.2.1 Dependency of abundance estimates on number of passes.

Capture probabilities were estimated, separately for the three different size groups, using captures from different numbers of passes (ranging from the first two passes to all available passes) to determine how estimated capture probability was dependent the number of passes used. All captured individuals (whether alive or dead) were used to derive estimates. Estimated abundances were then compared with total capture from all passes. Given that total capture from all passes was a conservative estimate of abundance (it is likely that most juveniles would have been captured from the large number of passes used), a comparison between the removal estimate and total capture provided an indication of whether the removal

180 estimate was over- or underestimating abundance. This comparison was conducted separately
181 for abundance estimates calculated using the first three passes, and abundance estimates
182 calculated using seven passes to determine bias associated with the number of passes used.
183 This enabled evaluation of whether using a greater number of passes than the traditional
184 three-pass approach would improve estimates. Seven passes were used, rather than the total
185 number of available passes (7-13 passes, dependent on survey), to ensure a consistent number
186 of passes used in the estimate.

187 2.2.2 Dependency of abundance estimates on use of block nets.

188 The probability of juveniles being captured in the block net rather than at the site of
189 application of the electrofishing gear was determined as a function of size group,
190 electrofishing pass and survey (including an interaction term between size group and
191 electrofishing pass and an interaction term between size group and survey) using stepwise
192 generalized linear modeling (binomial error distribution). For size groups, medium and large
193 individuals were pooled into one group to increase the group sample size. The potential effect
194 of using block nets on abundance estimates was then determined by comparing the abundance
195 estimate using all captured juveniles, both from electrofishing and the block net, with the
196 abundance estimate calculated using only the juveniles captured from electrofishing.

197 2.2.3 Electrofishing-induced mortality.

198 The probability of juveniles experiencing mortality was modelled as a function of size
199 group (small or medium/large), pass number and survey (including interactions between size
200 group and pass and between size group and survey) using stepwise generalized linear
201 modeling (binomial error distribution).

202 3. Results

203 3.1 Total captures and estimated capture probabilities

204 Total captures in all sites for Atlantic salmon and brown trout were always less than 2.5
205 individuals m^{-2} (the maximum capture occurring in Homla in 2010). Total captures of Atlantic
206 salmon were greater than brown trout, particularly in Homla, and in only two surveys (the
207 2010 surveys in Ingdalselva and Levangerelva) did brown trout abundance comprise
208 approximately a third of the total salmonid catch (Fig. 2). Overall, more small (< 60 mm)
209 Atlantic salmon juveniles were captured than medium-size (60-95 mm) juveniles. Large (≥ 95
210 mm) juveniles only constituted 15.1% of total Atlantic salmon capture. However, the size
211 class distribution of the captures varied according to site and year, and in some surveys more
212 medium-sized than small juveniles were captured.

213 The estimated Atlantic salmon capture probability (from all passes) varied greatly
214 according to site and year of surveying (Fig. 3). Estimated capture probability tended to
215 increase with size group, and in only one survey (Homla in 2015) was the capture probability
216 of the small size group greater than that of the large size group. Estimated capture
217 probabilities were not related to either salmonid density (the sum of all size groups for both
218 Atlantic salmon and brown trout) or temperature (Pearson's r , $p > 0.05$). For salmonid
219 density, correlations were 0.37 (small juveniles), 0.46 (medium juveniles) and 0.05 (large
220 juveniles). For temperature, correlations were 0.34 (small juveniles), 0.36 (medium juveniles)
221 and -0.22 (large juveniles). However, sample size ($N = 10$) was too small for us to be
222 confident that we were correct in accepting the null hypothesis of there being no relationship
223 between estimated capture probability and either salmonid density or temperature: assuming
224 an approximately normal distribution, this approach would require a correlation of 0.77 to
225 provide a hypothesis test with a Type I error of $\alpha = 0.05$ and a Type II error of $\beta = 0.2$ for $N =$
226 10.

3.2 Dependency of abundance estimates on number of passes

Estimated Atlantic salmon capture probability varied according to the number of passes that were used to derive the estimate (Fig. 4). This relationship also varied according to survey. Some surveys, for example Homla (2014), showed a rise in estimated capture probability with increasing number of passes used, whereas other surveys, for example Ingdalselva (2010) showed a reduction (Fig. 4a). The relationship between capture probability and number of passes was more consistent for large juveniles, with most surveys showing a decline in estimated capture probability with increasing number of passes. The relationship was more variable according to site for small juveniles (Fig. 4b). For example, capture probabilities estimated from three passes were much greater than those from seven passes in Homla (2010) and Ingdalselva (2013), whereas capture probabilities from three passes were less than those from seven passes for Homla (2014) and Vindøla (2010).

Estimated abundances were positively related to total capture from all passes, whether using the captures from the first three passes or captures from the first seven passes to derive the abundance estimate (Fig. 5). For medium and large juveniles, relationships between estimated abundances and total captures were stronger when estimates were derived from seven passes (medium juveniles, Pearson's $r = 0.97$, $p < 0.001$; large juveniles, $r = 0.98$, $p < 0.001$) rather than three passes (medium juveniles, $r = 0.96$, $p < 0.001$; large juveniles, $r = 0.92$, $p < 0.001$). For the small juveniles the relationship was actually weaker when using more passes to derive the estimate (three-pass, $r = 0.96$, $p < 0.001$; seven-pass, $r = 0.85$, $p = 0.013$): the relative weakness of this relationship was caused by two surveys (Homla 2010 and Ingdalselva 2013) where capture probabilities declined with successive pass, inflating the abundance estimate. Estimated abundance using captures from the first three passes tended to be lower than the estimates using seven passes, particularly for large juveniles.

3.3 Dependency of abundance estimates on use of block nets

All juveniles captured in block nets were found in the downstream rather than the upstream net. The probability of being captured in the block net rather than at the electrofishing gear was greater for small rather than medium/large individuals (Table 2). Overall, the probability of being captured in the block net increased with increasing pass number. Of total capture per pass, the proportion of juveniles captured in the block net, as opposed to being captured during electrofishing, varied greatly according to survey (Fig. 6a). For example, in Homla, the proportion was much higher in 2014 than in 2015. Including the counts of juveniles entrained in the block nets had a large effect on the abundance estimates in all surveys other than Homla (2015) (Fig. 6b). In all cases, the omission of net captures resulted in a reduction in estimated abundance. This effect was much greater for small than large juveniles, whether estimates were from all passes (reductions of 3.3-68.7%, 0-33.3% and 1.6-7.7% for small, medium and large juveniles respectively) or three-passes (reductions of 3.6-30.2%, 0-26.0% and 3.1-9.8% for small, medium and large juveniles respectively). There was a large variation in the relative reduction in estimated abundance according to survey. For example, the relative reduction in Homla (2015), where few individuals had been captured in the block net, was smaller than in Homla (2014) where more individuals had been captured in the block net. The relative reduction was generally greater when the abundance estimate was obtained from all passes rather than the first three-passes.

3.4 Electrofishing-induced mortality

Most dead fish were captured in the downstream block nets, few were captured away from the nets, and none were captured in the upstream nets. Dead fish captured at the block net were impinged on rather than gilled in the net. Total mortality varied greatly according to survey, being much greater in Homla (2015) (41.5%, 23.5% and 16.7% mortality among small, medium and large juveniles respectively) than in Ingdalselva (2013) (6.3%, 0.8% and

0% mortality respectively) or Toåa (2014) (8.6%, 0.8% and 0% mortality respectively). No mortality was observed in Homla (2014). Mortality probability was greater for small than medium/large juveniles (Table 3). This was particularly the case for Ingdalselva (2013) and Toåa (2014) where small individuals were particularly more likely to experience mortality (although the interaction terms retained during stepwise elimination were non-significant). No significant relationship existed between mortality probability and pass number.

4. Discussion

This study has shown that the key assumption of removal methods used in producing multi-pass electrofishing estimates – that capture probability stays constant between passes – may not always be true. Estimated capture probability depended on the number of passes used, with the change in estimate with successive pass depending on survey and size group. Given this, the traditional three-pass approach may bias the estimate of the population abundance and size (and consequently age) distribution. This study has also shown that the use of block nets, by preventing emigration of fish, may greatly alter abundance estimates and the estimated size or age distribution of the population, and that electrofishing mortality may be a pertinent issue.

Estimated Atlantic salmon capture probability (using all passes) was not related to either salmonid density or water temperature within each survey. Relationships established between capture probability and fish density in the literature have not been definitive. Korman et al. (2009), for example, found variable effects of density on capture probability of rainbow trout, *Oncorhynchus mykiss*, depending on habitat properties and the removal method used. Niemelä et al. (2000) found a weak negative relationship between capture probability and salmonid abundance. Speas et al. (2004) in contrast found a positive relationship between capture probability and brown trout abundance. Relationships between capture probability and temperature in the literature have likewise been inconsistent. Millar et al. (2016) attributed higher capture probabilities during summer to higher water temperatures. However, temperature effects have often not been detected (e.g., Bayley and Austen, 2002; Speas et al., 2004; Price and Peterson, 2010). In the current study, it is not possible to rule out the effect of salmonid density or temperature, given the small sample size and the fact that there may have been other confounding factors. However, the lack of a relationship between estimated capture probability and either salmonid density or temperature within the current study suggested that variation in these among surveys was not causing a bias in abundance estimates.

The capture probability of large juveniles was generally greater than that of small juveniles. This is consistent with previous work on salmonids that has found higher catchability in large individuals (Borgstrøm and Skaala, 1993). Electrofishing is more effective at immobilizing larger individuals (Dolan and Miranda, 2003). Additionally, larger individuals are also easier to spot, and may potentially make less use of interstitial spaces so may be easier to capture (Korman et al., 2009). The proportionally greater level of small compared to large juveniles captured in the block net rather than at the application of the electrofishing gear indicates that electrofishing may be less effective for small juveniles such as young-of-the-year. This may have resulted from stunned and dead juveniles of small size being displaced downstream without being observed, or stunned and surviving juveniles migrating downstream and subsequently impinging on the block net. The spatial and temporal variation revealed in this study indicates that monitoring of the abundance of young-of-the-year might be too methodologically constrained for electrofishing (but see Vehanen et al., 2010).

4.1 How many passes should be used?

This study has shown that the traditional multi-pass approach that involves just three passes may produce inaccurate estimates of both overall fish abundance and the population body size (and therefore age) distribution. As fish are removed in successive passes in multi-pass electrofishing, fish abundance (and therefore density) in the fished area declines. This reduction in density may make it more difficult to capture fish. Capture probability, therefore, may decline with increasing pass, so a three-pass approach would only be calculating relatively high capture probabilities and thus underestimating population abundance. For example, abundances estimated from the first three passes in the current study tended to be 10-20% less than those estimated using seven passes. How important this will be in terms of analyzing a fish population will depend upon the specific objective of the analysis. Several authors have shown that single pass electrofishing may provide adequate information (Kruse et al., 1998; Arnason et al., 2005; Bateman et al., 2005; Sály et al., 2009), but if the intention is to use the data for monitoring population abundances, a multi-pass approach involving a similar number of passes to that used in this study may be warranted. It should be noted, however, that using a large number of passes may not always be a perfect solution. In the current study, low capture probabilities were estimated for small juveniles in two surveys when using seven passes (Homla 2010 and Ingdalselva 2013), potentially resulting in an overestimate of abundance (which reduced the strength of the relationship between abundance estimate and total capture among all surveys; see Fig. 5).

Mortality did not consistently increase with the number of passes used in the current study, so such a multi-pass approach need not necessarily detrimentally impact the fish population. However, a multi-pass approach is resource intensive. In field surveys conducted by researchers in this study, a single electrofishing pass took two researchers ≈ 30 -40 min to complete for a 100 m² station (although the time required depended on fish abundance and habitat characteristics). Juvenile abundance at a station could be adequately surveyed within ≈ 1 h using a single-pass approach and ≈ 2 -2.5 h using a three-pass approach. Using a three-pass approach in three sites, or a single-pass approach in nine sites, may potentially provide more information on the fish population than using a nine-pass approach in one site; for example, giving information on the spatial distribution of the population. If one may assume that capture probability is relatively similar in one river on one sampling date, a combination of many passes at several sites (to establish the “correct” capture probability) with single-pass at most sites may provide reliable data for the population in that river.

4.2 Should block nets be used?

The installation of block nets may be used to ensure a closed population, meeting one of the assumptions of removal methods, and producing a more accurate abundance estimate. However, block nets have the disadvantage that they require effort and time to install that could otherwise be used in electrofishing. In the current study, installation of block nets took several hours, which would be enough time for an additional site to be surveyed. Additionally, the installation of block nets will also cause habitat disturbance which may initiate fish emigration. It is therefore debatable whether the added effort is justified. A greater proportion of small compared to medium and large juveniles was found in the block nets, possibly because it was harder to observe the smaller fish within the water. Block nets may therefore have more utility in surveys designed to obtain an accurate body size or age distribution of the population. However, habitat characteristics in some salmon rivers do not allow the easy installation of block nets. Based on the current study, the percentage reduction in estimate abundance ranged between $\approx 3\%$ and $\approx 30\%$ for the small size group using three-pass electrofishing, so in rivers where block nets cannot be used, this bias will not be negligible.

4.3 Mortality

Mortality was likely to have resulted from the direct effect of the applied electric field because juveniles were impinged upon but not gilled in the net. That is, there is no evidence to suggest that nets were causing mortality. Mortality was size-specific, being greater for small juveniles. Mortality is generally expected to be greater for larger individuals because the voltage differential across the fish body increases with size, but the effect of body size on mortality of fish undergoing electrofishing has proven inconsistent (see review by Snyder, 2003), and this effect is species-dependent (Dolan and Miranda, 2004); for instance, higher mortality has been observed in smaller individuals of cyprinids (Janáč and Jurajda, 2011). Field observations in the current study also indicated that some stunned small juveniles remained under stones in the substratum without being detected. These juveniles may subsequently have been killed by repeated electroshocks. To minimize mortality, it is thus important to use electrofishing gear which allows adjustment of voltage relative to water conductivity, or to make manual adjustments based on field measurements.

The sample size of surveys including data on mortality was too small ($N = 4$) to establish a statistical relationship between mortality and temperature. However, mortality was much greater in the Homla (2015) survey than in the other surveys. The Homla (2015) survey occurred when water temperature (18.3°C) was higher than in the other surveys – Homla (2014) 1.3°C , Ingdalselva (2013) 4.6°C and Toåa (2014) 13.9°C – which may be anecdotal support for a temperature effect on mortality. Electrofishing during conditions of high temperature may be stressful to fishes, and may result in injury (see for example Culver and Chick, 2015). Firstly, salmonids may be stressed in high temperatures. The standard metabolic rate of salmonids increases with temperature, increasing oxygen demand, while the level of dissolved oxygen in the water tends to decrease with temperature (Barnes et al., 2011). Temperatures as high as $\approx 18^{\circ}\text{C}$ are approaching critical incipient temperatures for Atlantic salmon of $22\text{--}28^{\circ}\text{C}$ (Elliott and Elliott, 2010). Secondly, fish electrical resistance is negatively related to temperature (Whitney and Pierce, 1957), so it is possible that a given electrofishing voltage may impart additional stress on the fish during high temperatures. We therefore suggest that researchers should be aware of this risk when conducting electrofishing salmonid surveys during high temperatures (e.g. above 18°C).

No consistent relationship was found between the probability of mortality and the number of electrofishing passes applied. This is somewhat surprising, as we would expect that repeated electrofishing passes covering the full area enclosed by the block nets would expose the fish remaining after one pass to further shocks during following passes. If the lack of a relationship found in this study represents a true absence of a relationship, this suggests that the application of multi-pass electrofishing should not be precluded on account of potential mortality. However, it should be noted that mortality may have been underestimated, particularly for small individuals, if dead individuals remained hidden in the substrate interstitial spaces.

5. Conclusion

Back-pack electrofishing is a convenient and often the most practicable method for sampling salmonid fishes in streams and small rivers. However, estimates derived from this method have to be handled with care. Based on our analysis of seven passes, we conclude that the standard method of three-pass removal will produce biased estimates of fish abundance. Firstly, traditional three-pass estimates may overestimate capture probability, causing an underestimate of population size, due to the fact that capture probability is higher in initial passes. This effect may be size-specific (occurring more for small than for large fish in the current study), meaning that the body size and age distribution of the population will be poorly estimated. Secondly, if block nets are not installed, there is the potential for migration

out of the electrofishing site, which may further bias estimates, particularly for those of the size and age distribution of the population. This bias may be further increased due to electrofishing mortality if dead juveniles drift downstream out of the electrofishing site without being observed if block nets are not present – mortality was greater for small juveniles in the current study meaning that there was a greater potential bias for this group. In general, abundance estimates of small juveniles (< 60 mm) based on the removal method can be highly inaccurate and must therefore be treated with care.

Mortality may increase substantially when water temperature is high. These factors mean that for the body sizes of young-of-the-year salmonids in low-productivity waters, electrofishing may be unreliable for estimating population densities, and may be better restricted to other sampling purposes. Consequently, electrofishing for juvenile Atlantic salmon at high water temperatures (> 18 °C) should be avoided in the interests of animal welfare. Combined with the recommendations from the European Committee for Standardization (Anonymous, 2003), stating that quantitative electrofishing for salmonids should not be performed at low temperatures (< 5 °C), a rule-of-thumb could be that quantitative electrofishing for juvenile salmonids is advised only for use at intermediate water temperatures.

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Figure captions

Figure 1. Surveyed rivers (upper panel) and site hydromorphological characteristics in September 2010 (lower panel). Substrate size categories are: sand (< 2 mm), gravel (2-19 mm), pebbles (20-99 mm), cobbles (100-250 mm), and boulders (> 250 mm). Shelter capacity was calculated according to the method outlined in Finstad et al. (2007), classified as small, medium or large.

Figure 2. Total captures from all passes (at the electrofishing gear and in the block nets) of small (< 60 mm), medium (60 - 95 mm), and large (\geq 95 mm) Atlantic salmon and brown trout juveniles for the ten surveys. Total captures are expressed per unit area. Captures for Atlantic salmon and brown trout are indicated by abbreviations "S" and "T" beneath the bars. Total number of passes are indicated above the bars.

Figure 3. Estimated capture probability (\hat{p}) of small, medium and large Atlantic salmon juveniles estimated using the Carle and Strub removal method based on captures from electrofishing and block nets (all passes used). Whiskers extent 1 SD above the estimate.

Figure 4. Effects of number of passes on the capture probability estimate (\hat{p}): (a) estimated capture probability for small, medium and large Atlantic salmon juveniles as a function of number of passes for two selected surveys; (b) ratio of estimated three-pass capture probability to seven-pass capture probability. In (b), the dotted lines show equivalent three-pass and seven-pass capture probability estimates.

Figure 5. Estimated abundance (\hat{N}) for small, medium and large Atlantic salmon juveniles using the first three passes and using seven passes versus total capture in all passes (7-13, according to survey; Table 1) for the respective size group. The dotted line shows equivalent abscissa and ordinate values.

Figure 6. Effect of use of block nets: (a) percentage of total capture captured in block nets as a function of size group and pass number; (b) percentage reduction in capture estimate resulting from ignoring individuals captured in block nets, using all passes and the first three passes.

Suppl. Figure 1. Length distribution of all captured Atlantic salmon juveniles.

Suppl. Figure 2. Estimated Atlantic salmon capture probabilities and abundances from different removal methods (Zippin, Carle and Strub, Moran, Schnute) using captures from all passes. Whiskers extend 1 SD above the estimate. The Schnute method estimates two capture probabilities: one capture probability for the first pass and another capture probability for all subsequent passes.

Table 1. Electrofishing surveys, showing number of Atlantic salmon juveniles captured and station properties.

River	Date	Nr. Passes	Capture in final pass	Total capture (all passes) per size group			Temp. (°C)	Area (m ²)	Length (m)
				Small	Medium	Large			
Homla	2010-Sep	12	5	294	118	40	12.0	220	13
	2010-Nov	10	27	160	79	76	4.3	357	19
	2014-Nov	8	1	46	100	60	1.3	190	17
	2015-Aug	10	10	414	85	60	18.3	329	26
Ingdalselva	2010-Sep	10	6	146	28	96	11.6	850	74
	2013-Oct	7	50	192	362	61	4.6	850	74
Levangerelva	2010-Sep	13	3	154	170	40	12.2	283	23
Toåa	2010-Sep	11	5	46	170	37	10.2	427	14
	2014-Sep	9	6	162	131	29	13.9	243	27
Vindøla	2010-Sep	10	5	43	188	52	10.5	450	23

Table 2

Table 2. Relationship between observed probability of being captured in the block net (rather than at the electrofishing gear) and size group (small or medium/large), ~~and~~ pass number ~~and survey~~ established using stepwise generalized linear model~~ings~~ (family = binomial). The estimate shown is the expected value.

	<u>Estimate</u>	<u>S.E.</u>	<u>z value</u>	<u>Pr(> z)</u>	<u>Odds ratio</u>
<u>(Intercept)</u>	<u>-2.889</u>	<u>0.246</u>	<u>-11.751</u>	<u><0.001</u>	<u>0.056</u>
<u>Size group (small)</u>	<u>1.106</u>	<u>0.152</u>	<u>7.302</u>	<u><0.001</u>	<u>3.023</u>
<u>Pass</u>	<u>0.232</u>	<u>0.034</u>	<u>6.768</u>	<u><0.001</u>	<u>1.261</u>
<u>Survey (Homla 2015)</u>	<u>-2.395</u>	<u>0.358</u>	<u>-6.688</u>	<u><0.001</u>	<u>0.091</u>
<u>Survey (Ingdalselva 2013)</u>	<u>0.800</u>	<u>0.247</u>	<u>3.246</u>	<u>0.001</u>	<u>2.227</u>
<u>Survey (Toåa 2014)</u>	<u>0.133</u>	<u>0.272</u>	<u>0.487</u>	<u>0.626</u>	<u>1.142</u>

Survey	Parameter	Estimate	S.E.	z-value	Pr(> z)	Odds ratio
Homla (2014)	(Intercept)	-2.708	0.327	-8.292	<0.001	0.067
	Small size group	1.881	0.458	4.112	<0.001	6.562
Homla (2015)	(Intercept)	-4.97	1.003	-4.953	<0.001	0.007
	Small size group	1.617	1.040	1.556	0.120	5.040
Ingdalselva (2013)	(Intercept)	-1.800	0.204	-8.83	<0.001	0.165
	Pass	0.171	0.046	3.697	<0.001	1.187
	Small size group	0.949	0.188	5.042	<0.001	2.584
Toåa (2014)	(Intercept)	-3.896	0.426	-9.155	<0.001	0.020
	Pass	0.523	0.075	6.961	<0.001	1.687
	Small size group	1.399	0.366	3.823	<0.001	4.052

Table 3

Table 3. Relationship between observed mortality probability and size group (small or medium/large) ~~and~~ pass number and survey established using stepwise generalized linear modelings (family = binomial). The estimate shown is the expected value.

		Estimate	S.E.	z value	Pr(> z)	Odds ratio
(Intercept)		-1.344	0.205	-6.554	<0.001	0.261
Stage (small)		1.002	0.228	4.396	<0.001	2.725
Survey (Ingdalselva 2013)		-3.598	0.615	-5.855	<0.001	0.027
Survey (Toåa 2014)		-3.725	1.024	-3.639	<0.001	0.024
Stage (small) × Survey (Ingdalselva 2013)		1.231	0.690	1.784	0.074	3.426
Stage (small) × Survey (Toåa 2014)		1.708	1.066	1.603	0.109	5.520
Survey	Parameter	Estimate	S.E.	z-value	Pr(> z)	Odds ratio
Homla (2015)	(Intercept)	-1.344	0.205	-6.554	<0.001	0.261
	Small-size-group	1.002	0.228	4.396	<0.001	2.725
Ingdalselva (2013)	(Intercept)	-5.980	1.407	-4.251	<0.001	0.003
	Pass	0.274	0.296	0.924	0.355	1.315
	Size-group (small)	4.421	1.515	2.919	0.004	83.185
	Pass × small-size-group	-0.626	0.343	-1.824	0.068	0.535
Toåa (2014)	(Intercept)	-6.213	1.110	-5.598	<0.001	0.002
	Pass	0.350	0.105	3.328	0.001	1.418
	Small-size-group	2.672	1.049	2.547	0.011	14.463

Figure 1
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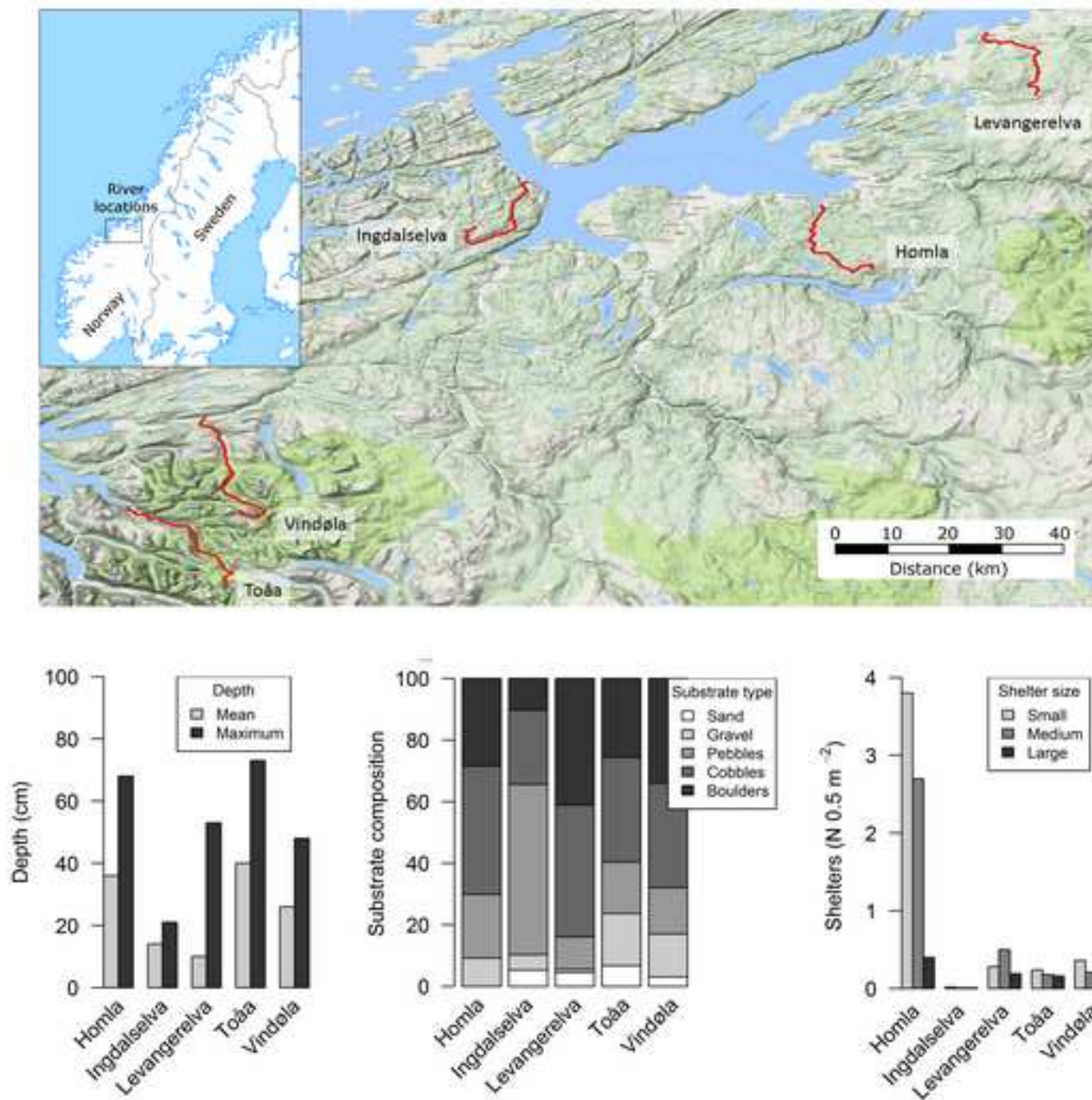


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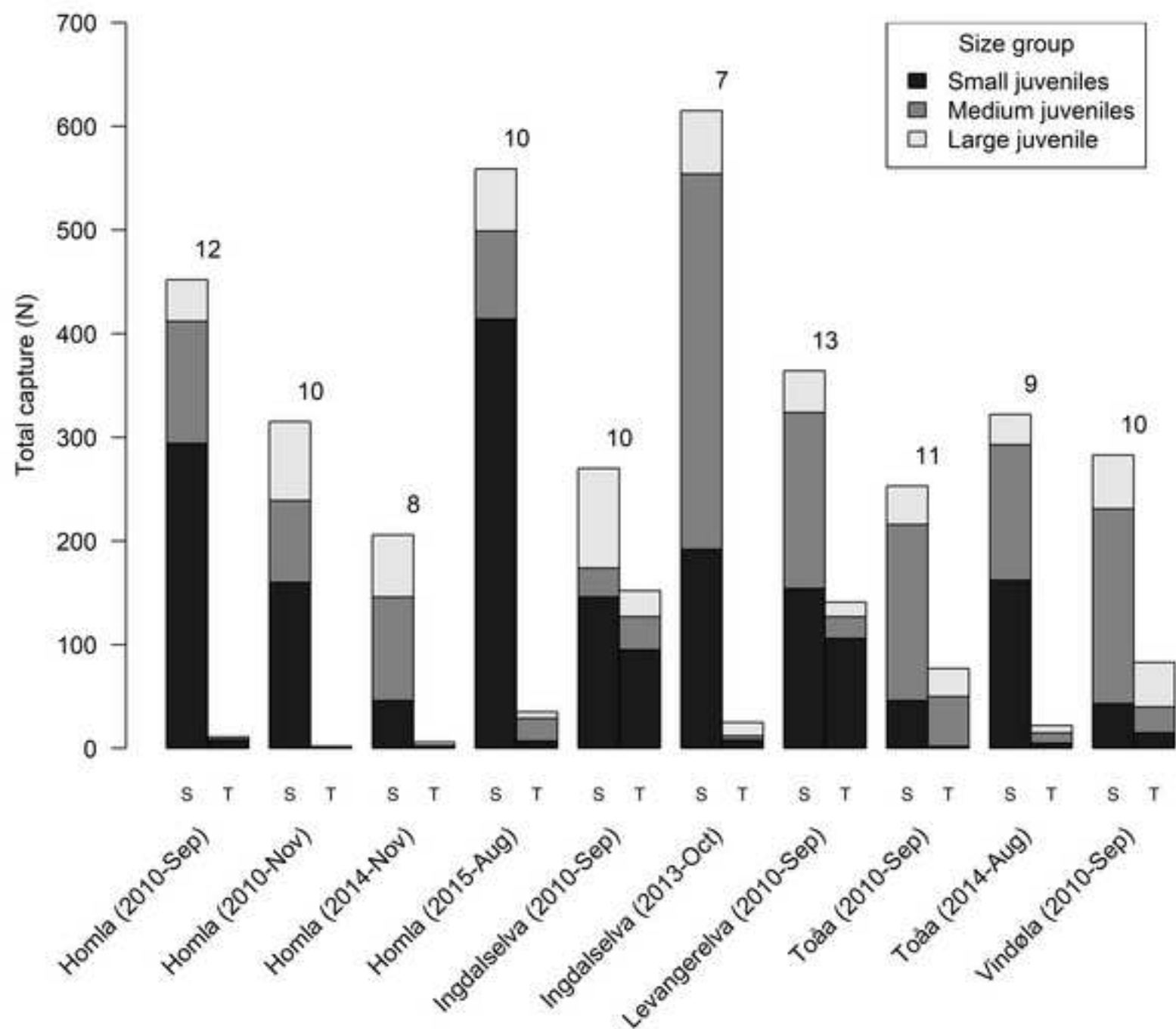


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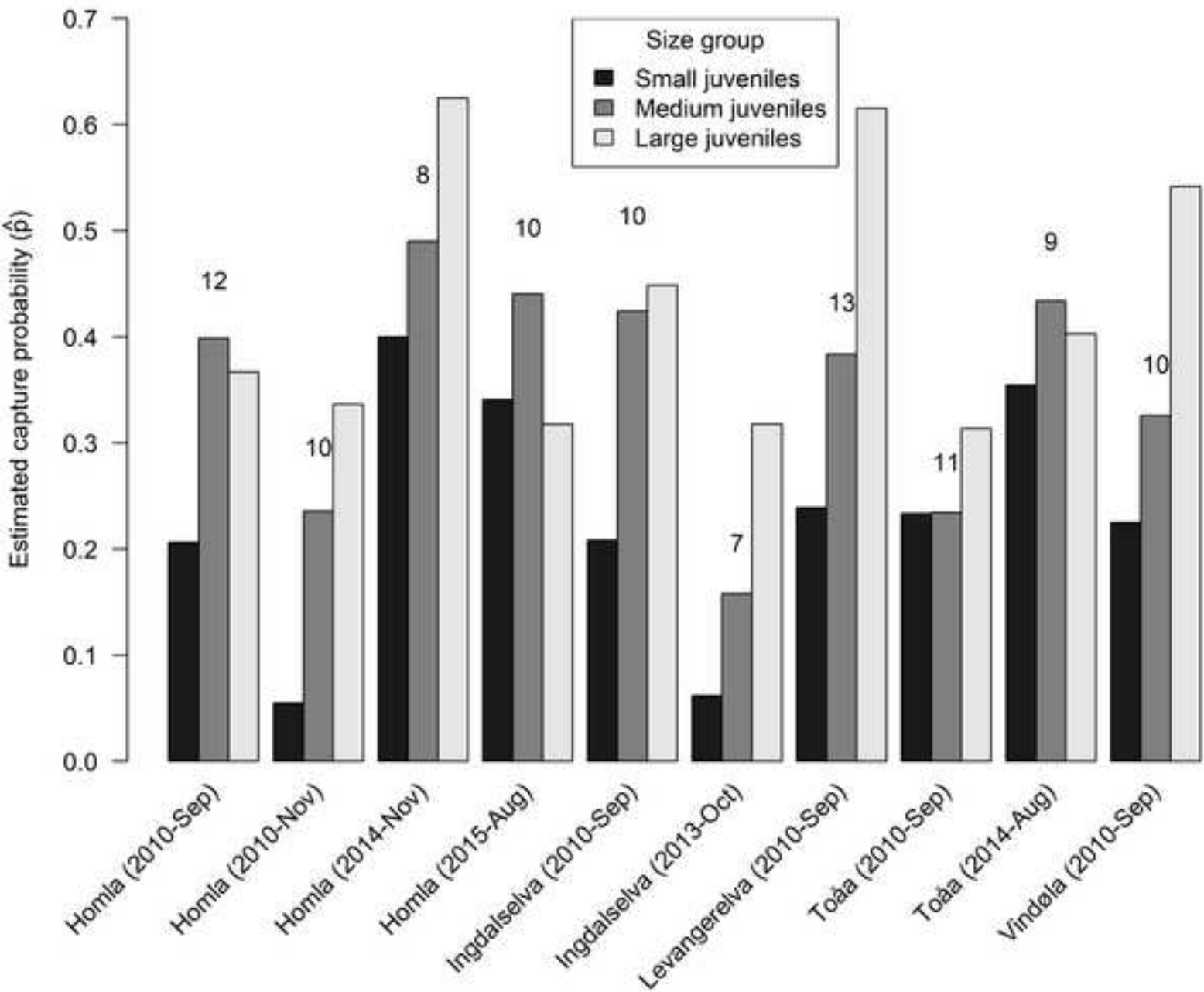


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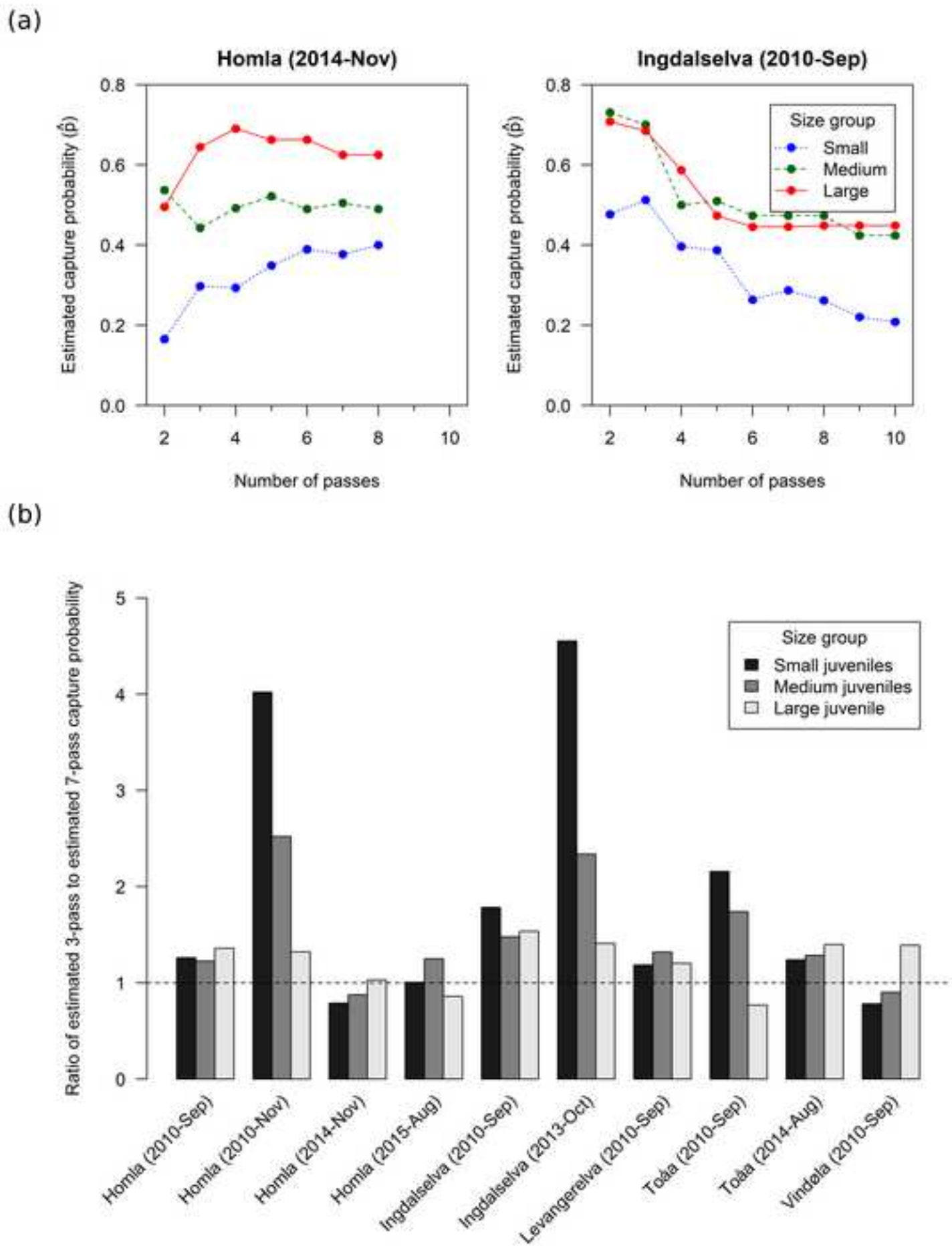


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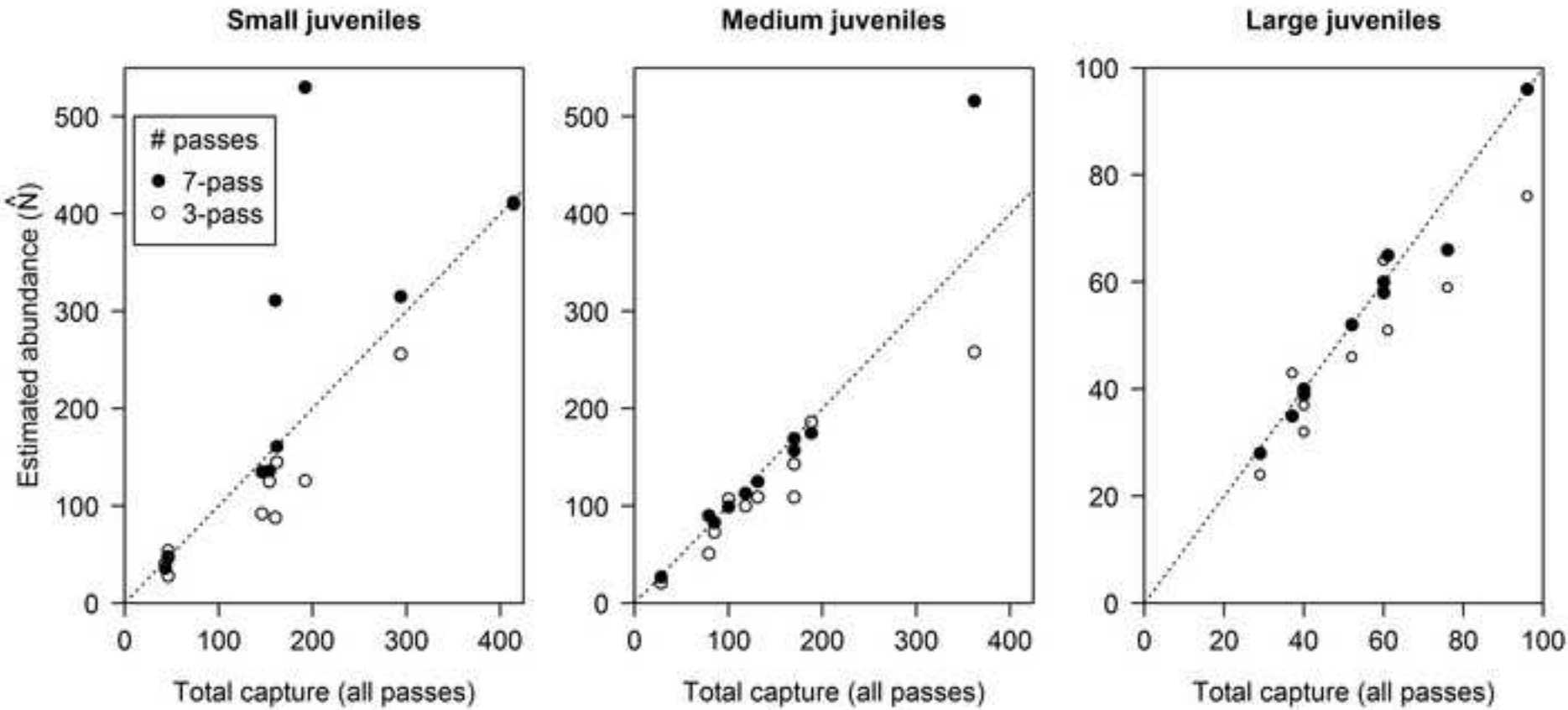


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