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Can structural enrichment reduce predation mortality and increase recaptures of hatchery-reared Atlantic salmon Salmo salar L. fry released into the wild?

--Manuscript Draft--

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Abstract:	Captive-reared fish often have poor survival in the wild and may fail to boost threatened populations. Enrichment during the nursery period can in some circumstances generate a broader behavioural repertoire than conventional hatchery production. Yet, we do not know if enrichment promotes survival after release into the wild. We conducted a field experiment during three field seasons using 0+ Atlantic salmon Salmo salar L. to investigate if enrichment during rearing, in the form of structural complexity (shelters), reduced immediate (within 2 days after release) predation mortality by piscine predators (brown trout Salmo trutta L.) and if such rearing environments improved long-term (2-3 months after release) post-release survival. In addition, we investigated if predation mortality of released fry was size-selective. S. salar fry were reared in a structurally enriched environment or in a conventional rearing environment and given otolith marks using alizarin during the egg stage to distinguish between enriched- and conventionally-reared fry. The outcome from the field experiments showed that structural enrichment did not consistently reduce immediate predation mortality and it did not improve, -or had a negative effect on, the recapture rate of fry from the river 2-3 months after release. The data also showed that enriched rearing tended to reduce growth. Additionally, we found that S. trutta predators fed on small individuals of the released fry. Overall, the data suggest that structural enrichment alone is not sufficient for improving long-term survival of hatchery-reared fish after release, and that other factors might affect post-release survival.

<u>Ethics Questionnaire for JFB</u>

Submitted manuscripts will only be considered if the experimental methods employed are ethically justified. Please answer <u>all</u> questions. If you have answered 'yes' to questions 4 to 7, you should include an *Ethics* paragraph in the Methods section of your manuscript which justifies your methods used. You should complete this questionnaire based on <u>all</u> fishes used in your experiment. For example, if you used live fishes as prey in predation experiments, this is a lethal endpoint for the prey fish (see Questions 5 & 6). Please read the Editorial published in JFB **68**, 1-2, for full information on JFB ethics. PLEASE SUBMIT THE COMPLETED QUESTIONNAIRE WITH YOUR MANUSCRIPT ONLINE THROUGH EDITORIAL MANAGER.

Corresponding author's name: **Anne Gro Vea Salvanes**

Question 1: Were fishes collected as part of faunal surveys? **YES**

If 'Yes', have the fishes, where feasible, been killed rapidly or returned to the wild after being held in aquaria and have procedures complied with local and or national animal welfare laws, guidelines and policies? If Yes, state these and provide suitable evidence (e.g. for the U.K. a Home Office PPL number is sufficient) that protocols have undergone an ethical review process by an institutional animal care and use (or similar) committee, a local ethics committee

The experiments have undergone ethical review by the Norwegian Food Safety Authority and are in terms with "The Regulation on the use of animals in research": Appoval FOTS id 8706. Most predators caught in this experiment were either anaesthetized with metacain (MS222) to enable evacuation of the stomach contents. After the procedure these fish were housed in 10 L containers to recover, before they were released back into the river. Some trout predators were euthanized by an overdose of metacain. All resamples of released salmon fry were rapidly killed by an overdose of metacain.

Question 2: If you have undertaken experimntal work, has the care and use of experimental animals complied with local and or national animal welfare laws, guidelines and policies? **YES**

If 'Yes', state these and provide suitable evidence (e.g. for the U.K. a Home Office PPL number is sufficient), both here and in the manuscript, that protocols have undergone an ethical review process by an institutional animal care and use (or similar) committee, a local ethics committee, or by appropriately qualified scientific and lay colleagues.

All procedures have been completed according to the Norwegian Food Safety Authority in terms with “The Regulation on the use of animals in research” with FOTS id 8706. Most predators caught in this experiment were either anaesthetized with metacain (MS222) to enable evacuation of the stomach contents. After the procedure these fish were housed in 10 L containers to recover, before they were released back into the river. Some predators were euthanized by an overdose of metacain. All resamples of released salmon fry were rapidly killed by

If ‘No’, because these laws do not exist in your country, please state this. Alternatively, if you carried out purely observational work so ethical permission was not considered necessary please state this both here and in the manuscript.

Question 3: Were fishes killed during or at the end of your experiment (e.g. for tissue sampling)? **YES**

If ‘Yes’, what method was used? Please provide details both here and in the manuscript.

Yes. Some predators were euthanized with an overdose of metacain (MS222); see above.

Question 4: Have you performed surgical procedures? **NO**

If ‘Yes’, please give brief details of the surgery here. Full details should be given in the manuscript. If the procedures caused more than slight pain or distress, did you use appropriate sedation, analgesia and anaesthesia, with post-operative care? Please provide full details and justification both here and within the manuscript including type and

Question 5: Did you use experimental conditions that severely distressed any fishes involved in your experiments? **NO**

If ‘Yes’, state the conditions and how they can be justified. What humane endpoints were used to minimise the effects? Please provide full justification within the methods section of your manuscript.

Question 6: Did any of the experimental procedures, particularly those that involve lethal endpoints (e.g. predation studies, toxicity testing), cause lasting harm to sentient fishes? **NO**

If 'Yes', provide details both here and in the methods section of your manuscript. Normally these procedures will be considered unacceptable by JFB unless any harm caused can be justified against the benefits gained.

Question 7: Did any of your procedures involve sentient, un-anaesthetised animals paralysed by chemical agents such as muscle relaxants? **NO**

If 'Yes', provide details both here and in the methods section of your manuscript. Normally these procedures will be considered unacceptable by *JFB*.

To the Assistant Editor
Dr. Nina Jonsson
Journal of Fish Biology
London

Bergen 11th April 2019

Dear Dr. Jonsson

Resubmission of MS-19-0111: «Can structural enrichment reduce predation mortality and increase recaptures of hatchery-reared Atlantic salmon *Salmo salar* L. fry released into the wild?"

Thank you for the constructive feedback on the previous version of our paper "Can structural enrichment reduce predation mortality and increase recaptures of hatchery-reared Atlantic salmon *Salmo salar* L. fry released into the wild?", and for inviting us to resubmit. We have now revised the paper to meet the comments from the two reviewers. Enclosed please find both the final manuscript and also the same version with track changes. Below we describe the changes made.

Best wishes,
Anne Gro Vea Salvanes

On behalf of Martine Røysted Solås, Helge Skoglund and Anne Gro Vea Salvanes

Reviewer(s)' Comments to authors and our responses to them:

Major comments Reviewer 1

1. Release site details

   How many juvenile salmonids were already resident in the areas, and with the addition of the hatchery fish what did the addition of the hatchery fish push density up to? How complex was the habitat into which the animals were released and what was the substrate like? Did low complexity and high embeddedness result in a paucity of shelters? The paper said the stocking site was above the range of the habitat of anadromous salmon, yet Atlantic salmon was one of the predators present at the stocking site? Where did they come from? All of this needs to be explained to assist with interpretation of the results.

   We thank the reviewer for the comment and we have now added the information to clarify these matters (see lines 236-244).

Enrichment specification

2. *First, "enrichment" is a broad term and encompasses many different aspects. The MS tests one of many types of enrichment. The title of the MS is specific about the type of enrichment being used, but the abstract is not. I would add words in the abstract to explain that the enrichment treatment is limited to adding structural complexity/shelters to the rearing habitat, so the reader knows exactly what the article is about.*

   We agree with the reviewer and have added the missing clarification in the abstract (see line 40-41).

Specific comments

1. *l. 36 add "in some circumstances" before "can"*
   We have added "in some circumstances" after "can", as we found this to improve the message.

2. *l.36 Is flexible the right word? Do you mean "broader behavioral repertoire"?*
   We agree with the reviewer and have changed the wording according to the suggestion of the referee.

3. *l. 40 change "reduces" to "reduced"*
   Corrected

4. *l.42 change "can improve" to "improved"*
   Corrected

5. *l. 47 change "could" to "did"*
   Corrected

6. *l.49 change "show" to "showed"*
   Corrected

7. *l. 103 add "the" before "efficiency"*
   Corrected

8. *l. 106 add "High" before "Mortality"*
   Corrected

9. *l. 108 to read "…provide a homogenous environment typically lacking structure where…"*
   Corrected

10. *l. 109 change "suggest" to "suggested"*
   Corrected

11. *l. 110 What do you mean by "certain skills"? Please be more specific.*
   We apologize for the vague description and have now changed “certain skills” to “skills associated with survival” to clarify the meaning.

12. *l.114 change "question whether" to "hypothesize that"?*
   Corrected

13. *l.115 to read "inferior antipredator behaviour of released fish increases predation mortality and that predation is a major cause for the loss of hatchery fish liberated to the wild (……."*
   Corrected

14. *l. 117 change "of" to "needed by"*
   Corrected

15. *l. 119-120 to read "… Hatchery-reared and wild fish have similar reflex responses to threats, but hatchery individuals are seemingly less risk-averse…"*
   Corrected

16. *l. 122 to read " In fishes, escape from danger depends on swimming speed, which in turn is a function*
   Corrected

17. *l.123 to read "As a fish is growing, its number…."*
   Corrected

18. *l. 125-126 to read ""…., and also because predators become increasingly gape limited and unable to consume larger individuals…."*
   Corrected

19. *l. 128 change "and" to "or"*
   Corrected

20. *l. 131 add "such" before "as"*
   Corrected

21. *l. 132 change "flexible" to "diverse"?*
   Corrected

22. *l.137 change "using" to "subjected to"*
    Corrected

23. *l. 140 add "also" before "improve"*
    Corrected

24. *l.143 to read "….found enrichment impacts the development of foraging behaviour (refs) and reduces swimming activity…"*
    Corrected (we also added “that” between “found” and “enrichment”)

25. *l.52 change "off" to "of"*
    Corrected

26. *l. 156 strike the "the" before "behavioural"*
    Corrected

27. *l. 158 add "about" before "whether"*
    Corrected

28. *l. 162-163 "group marked" to be hyphenated to "group-marked"*
    Corrected

29. *l. 167-169 is confusing. How about " This was done by searching for released fry in the stomach contents of predators (primarily brown trout, Salmo trutta L.) resident at the release site. The predators were sampled 4 and 48 hours after the release of the fry."*
    Corrected. We have also made sure to add a “.” After “L” in the other cases where species name is mentioned.

30. *l. 172 add "ones" after "larger"*
    Corrected

31. *l. 174 to read "…. large individuals. This is especially true for piscine predators…."*
    Corrected

32. *l. 179 the term "behavioural flexibility" is vague. I think you mean have developed a suite of behaviour adapted to use shelter"*
    We apologize for the unclear sentence. We have now made some edits for clarification (see lines 181-183).

33. *l. 188 change "live" to "captive"*
    Corrected

34. *l. 195 change "was" to "were"*
Corrected

35. *l. 197 strike "and did not get a second treatment". You do not need this.*
Corrected

36. *l. 198 add "was not intrusive and" before "should not"*
Corrected

37. *l. 199 strike "according to" and the line to read "controls (Baer and Rosch, 2008)"*
Corrected

38. *l. 204 add "but similar" before "rearing"*
Corrected

39. *l. 209 I do not understand what is meant by "sheds" and the figure did not help. Did you mean strands?*
We thank the reviewer for spotting this typo. The typo is replaced with "shreds".

40. *l. 214 strike "with a few seconds intervals"*
Corrected

41. *l. 216 change "fungi" to "fungus"*
Corrected

42. *l. 217 change "made" to "resulted in"*
Corrected

43. *l. 227 change "on" to "in"*
Corrected

44. *l. 235 change "took" to "measured"*
Corrected

45. *l. 248 change "electro-fishers" to "electrofishing team". Electro-fishers are the machines!*
Corrected

46. *l. 266 change "content" to "contents"*
Corrected

47. *l. 259 add "a" before "gastric"*

    Corrected

48. *l. 264 change "digestion" to "decomposition". At this point digestion has terminated, probably.*

    Corrected

49. *l. 266 change "take out fish" to "lethally sample fish"*

    Corrected. We also changed "fish" to "predators" for clarification.

50. *l. 277 change "n=ca." to "about"*

    Corrected

51. *l. 282 to read "....0.01g). We only measured fry where the digestive processes had not proceeded to the point that length measures would be compromised. To ensure this, a scoring system was developed (Table 4) where each fish was scored for its state of digestion. For analysis, only lengths of fish which scored 0 were used."*

    We apologize for that the potential uncertainty in length measures were not expressed sufficiently clear in the previous MS version. This led the referee misunderstand slightly the meaning. We do therefore not agree fully with the suggested formulation from the referee. We have now edited the text to and hope our message is clearer (see lines 296-299).

52. *l. 293 This only applies to the fish that were lethally sampled?*

    We meant the released *S. salar* fry that were either consumed by predators or recaptured two-three months later. The text is now revised to clarify this (see line 310).

53. *l. 307 Need to explain what these additional fish are. The way the line is written, it suggests that there are other stocking programs underway into your watercourses. Is that true? Or are these resident trout fry? Does the presence of these fish compromise your interpretation of the significance of your results? Are they occupying all of the shelters so that there is no place for the enriched fish to go, hence explaining why you did not see a positive effect from your experimental treatments?*

    These additional fish are fish which age is ≥ 1 year, meaning they were released in preceding years. We added a sentence to clarified this in the text (see line 325). We do unfortunately not have information on the details regarding the presence of fish from earlier releases, and we are therefore not able to elaborate much on this topic.

54. *l. 324 change "test" to "tested", then strike "and in recaptured samples"*

    Corrected. However, since the chi-square test was used for recaptured samples also, we have edited the paragraph to include this information as well (see lines 340-342).

55. *l. 338 change "weeks" to "week"*

    Corrected

56. *l. 343 change "show" to "showed"*

    Corrected

57. *l. 354 Where did these Atlantic salmon come from? You stated the sites were above the anadromous salmon's distribution.*

    These are fish released in previous years (since 2013 there has been stocking of fish at both Rasdalen and Brekkhus) We have now added this information to in the text (see lines 242-244 and line 366).

58. *l. 360 The figure caption is not clear, hence I do not understand the figure and do not see how it supports this assertion. In the Figure caption what does "grey bars refer to distribution overlaps of the two"? Please clarify.*

    We agree with the reviewer that this Fig caption needs improvement. We have revised the figure according to the suggestion by the reviewer 2, and visualized the data in four separate panels instead of two and the figure caption is changed accordingly.

59. *l. 364 to read "… fry at both sampling times (4h and 48h) after fry…."*

    Corrected

60. *l. 365 add "h" after "4"*

    Corrected here and elsewere

61. *l. 382 to read "459 fry were recaptured…."*

    Corrected

62. *l. 393 add "for" before "all"*

    Corrected

63. *l. 395 strike the hyphen after "length"*

    Corrected

64. l. 396 strike the hyphen after "mass"

Corrected

65. l. 410 change "with" to "at"

Corrected

66. l. 411 change "fry to" to "fry from"

Corrected

67. l. 412 change "and" to "versus" and strike "just after fry release"

Corrected

68. l. 414 add "differing" after "two"

Corrected

69. l. 415 change "conducted" to "evaluated"

Corrected

70. l. 416 add "or not" after "Whether"

Corrected

71. l. 418 change both semicolons to commas

Corrected

72. l. 422-423 to read " "…to dominate smaller fish (Metcalfe…."

Corrected

73. l. 424 add "a" before "stress"

Corrected

74. l. 426 I would close this sentence up with the previous paragraph

Corrected

75. l. 427 strike the comma after "mortality"

Corrected

76. l. 428 strike "and that these were present in the release stretch at the time of predator sampling."

Corrected

77. l. 430 change "show" to "showed" and add "provided" after "and"

Corrected

78. *l. 432 change "show" to "showed" and change "days" to "sampling periods"*

Corrected

79. *l. 433 change "could" to "did"*

Corrected

80. *l. 435 strike "our hypothesis and" change "that might improve their ability to avoid predators" to "is not always true"*

The sentence is revised to clarify the message (see lines 447-449).

81. *l. 439 change "recaptures to "sampling periods"*

The text is now revised to clarify the message, end we include the change suggested by the referee.

82. *l. 441 change "one recapture" to "one site on one date"*

Corrected

83. *l. 442-443 Strike the sentence "Most of our results… into the wild."*

Corrected

84. *l. 446 add "primary" before "predator"*

Corrected

85. *l. 459 to read "…."It might be that there was an effect, but its impact was so small….."*

Corrected

86. *l. 460 add "to identify it" after "study"*

Corrected

87. *l.461 add "also" after "was"*

Corrected

88. *l. 462 to read "…. Stomach content data available consisted…"*

Corrected

89. *l. 465 Were your releases generating high densities at the site? How do you know? Also, change "several" to "some"*

We thank the reviewer for pointing this out. Earlier investigation of densities in other parts of the Vosso river have found the natural density of 0+ salmon to be between 10-40 ind./100m2. The density in the release stretch at the day of release was in our experiment 290 ind./100m2 and 160 ind./100m2 for the Rasdalen stretch and

Brekkhus stretch respectively. We have clarified this in the revised manuscript (see lines 477-482).

90. *l. 468 to read "… instead of actually sheltering…."*

Corrected

91. *l. 470 change "has been taken" to "becomes available"*

Corrected

92. *l. 486 and 487 strike "Jr."*

This paragraph has been removed after suggestion from reviewer 2, so this comment is no longer relevant, but we thank the reviewer for the reminder.

93. *l. 509-515 I would strike this paragraph. It does not add anything to the paper*

Corrected

94. *l. 523 add "in this study" before "this variation"*

Corrected

95. *l. 526 change "likely to believe" to "probable"*

Corrected

96. *l. 528 change "of" to "for"*

Corrected

97. *l. 532 change "increase" to "increases"*

Corrected

98. *l. 534 to read " … than smaller fish, and in"*

Corrected

99. *l. 549 change "have" to "has" and add "been" before "shown"*

Corrected

100. *l. 555 add "possible" before negative. Also, can you provide suggestions of what this negative effect was that could generate this result?*

Corrected. We have added a sentence about the negative result, which is also discussed later in the discussion (see lines 559-562).

101. *l. 560 add "to the present results" after "comparable"*

We thank the reviewer for the suggestion. However, this sentence has been changed to: "Although these experiments differ in species studied, salmonid life stage tested, quantity-, type-, and timing of enrichment provided during rearing, and sampling procedure, they show, together with our data reported here…" to meet the comment from reviewer 2 (see lines 566-569).

102. *l. 562 to read " ….that the benefits from enrichment on post-release survival are not…"*

Corrected

103. *l. 571 Groups? What groups? You lost me here.*

We apologize for the unclarity of this sentence and have now changed this to "treatment groups" and rewritten the sentence to make our points clearer (see line 577).

104. *l. 575- 576 to read '….tendency towards a differences in length between enriched fry and control fry on the day of release seemed to have been maintained at least at Rasdalen for 2-3 months for all years."*

Corrected

105. *l. 578 strike "at the last day of rearing"*

Corrected

106. *l. 579-580 to read " Perhaps the size of the released fish was a more important factor for survival over time"*

Corrected

107. *l. 581 change "obtain" to "obtained"*

Corrected

108. *l. 584 to read. "….maybe the enrichment treatment could have shown beneficial effects if we…."*

Corrected

109. *l. 586 change "long" to "longer"*

Corrected

110. *l. 591 change the hyphen to a comma*

Corrected

111. *l. 592 to read "…cannot provide a categorical conclusion on whether…."*

Corrected

112. *l. 610 to read "type in combination with other factors should be used when…."*

Corrected

113. *l. 700 and 703 strike "Jr."*

The paragraph where these references are used has been removed after suggestion from reviewer 2, but we thank the reviewer for the reminder.

114. *Table 1 and Table 3 can be combined into a single table.*

Corrected

115. *Fig 1 The photographs are difficult to see details in, and will not print well. If better pictures are not available, it might be better to do a drawing of the apparatus or strike the figure.*

New illustrations have been made.

116. *Fig. 2. The resolution needs to be sharpened.*

Corrected

Specific comments Reviewer 2

1. Line 196 - The author refers to a paper on this marking technique, but maybe it is a good idea to write here what the outcome of the marking is (one ring for control and two rings for enriched in the otoliths?)

   We have now added a sentence to clarify this (see lines 203-204).

2. Line 228 - unclear sentence. Do you mean "migration obstacle preventing the wild population to reach this area"?

   Yes, and the sentence is now corrected.

3. Line 241 - How long is "a short period"? Clarify

   We thank the reviewer for spotting this unclear phrase. We have now edited the text to clarify the procedure we used (see lines 250-253)

4. Line 304 - I think you should also give the library used for the statistics, and not only for the graphs

We have added information about the libraries used in our statistical analysis (see lines 323-324)

5. Line 316-317 and elsewhere - do you mean two-tailed?

   We thank the reviewer for pointing this typo. The text is corrected.

6. *Line 330 - can you really justify using a one-sided (one-tailed?) test here, compared to the two-tailed ones elsewhere?*

   We thank the reviewer for pointing this out to us. We have now changed this to be a two-tailed test and the results have been adjusted accordingly. All Kolmogorov-Smirnov tests were still significant (although only weakly significant for the enriched group in 2017 – this has been pointed out in the result text). We have also removed "two-tailed" before "KS-test" in the result section since all KS-test now are two-tailed. The discussion section on size-selective mortality remains the same.

7. Line 376-377 - this is unclear. Instead: The fry consumed were smaller than average, in both treatment groups.

   We apologize for the unclarity of this sentence. However, we believe that when we are referencing the Kolmogorov-Smirnov test results we cannot refer to average values, as this test compares size distribution. We have rewritten the sentence to make our points clearer (see lines 388-393).

8. Line 392 - I think you mean that the distribution was wider (or that the size range was wider)
   Yes. Corrected.

9. Line 404 - change: The condition factor ……..was higher….
   Corrected here and elsewhere.

10. Line 411 - replace "fry to" with "fry from"
    Corrected

11. Line 418 - replace semicolons with colons (just a typo I guess)
    We have replaced the semicolons with commas, which was suggested by reviewer 1.

12. *Line 482-489 - The discussion is very long, and you should concentrate on the relevant issues for this study. The coloration has not been studied here, and there is no reason to believe that the fry from the different treatments should differ in coloration. I suggest you remove this paragraph.*
    We take the point and have removed this paragraph in the revised manuscript.

13. Line 580 - remove "just as, if not" - reads easier as: have been an even more important factor for survival.....
    We thank the reviewer for the suggestion. However, this sentence has been changed to: “Perhaps the size of released fish was a more important factor for survival over time, when both rearing treatments obtained experience in the wild.”, which was suggested by reviewer 1 (see lines 585-586).

14. Line 559-560 - remove "not directly comparable due to that"
    Corrected

15. Fig 4 - I think the histograms would be clearer if you have separate bars for predators that had eaten fry and those that had not. The overlap bars are not clear to me. For example, at the highest "overlap" bar, is the number 5 for non-consumers and 6 for consumers? Just think about it anyway.....

    We agree with the reviewer and have now edited the figure so the data are visualized in four separate panels instead of two. The figure caption has been edited accordingly.

16. Fig. 3 and 5 - I think this would be clearer with ordinary histograms (separate for treatments)

    We disagree with the reviewer on this point. We found differences between the distributions to be more clearly visualised by cumulative distributions. However, if the editor decides that histograms should be used, we will make the necessary changes.

UNIVERSITY OF BERGEN
Department of Biological Sciences

SIGNIFICANCE STATEMENT

Laboratory experiments report that the use of enrichment during rearing of fish might improve behavioural repertoire and that it supposedly could increase their post-release survival. Yet, there is limited knowledge about its effects after release into the wild. The field experiment reported here suggests that structural enrichment alone might not be sufficient to improve survival.

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REGULAR PAPER

Can structural enrichment reduce predation mortality and increase recaptures of hatchery-reared Atlantic salmon *Salmo salar* L. fry released into the wild?

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ABSTRACT

We conducted a field experiment during three field seasons using age 0+ year Atlantic salmon *Salmo salar* to investigate if enrichment during rearing, in the form of structural complexity (shelters), reduced immediate (within 2 days after release) predation mortality by piscine predators (brown trout *Salmo trutta*) and if such rearing environments improved long-term (2–3 months after release) post-release survival. In addition, we investigated if predation mortality of released fry was size-selective. *Salmo salar* fry were reared in a structurally enriched environment or in a conventional rearing environment and given otolith marks using alizarin during the egg stage to distinguish between enriched and conventionally-reared fry. The outcome from the field experiments showed that structural enrichment did not consistently reduce immediate predation mortality and it did not improve, or had a negative effect on, the recapture rate of fry from the river 2–3 months after release. The data also showed that enriched rearing tended to reduce growth. Additionally, we found that *S. trutta* predators fed on small individuals of the released fry. Overall, the data suggest that structural enrichment alone is not sufficient to improve long-term survival of hatchery-reared fish after release and that other factors might affect post-release survival.

KEYWORDS

conservation, enriched rearing, fish stocking, predation mortality, *Salmo salar*
size-selectivity

1 | INTRODUCTION

Release of captive-reared fish to supplement reduced wild populations has become a tool in conservation and management of fish populations (Salvanes, 2001). The released fish do, however, often suffer from high mortality rates after release (Henderson & Letcher, 2003; Sparrevohn & Støttrup 2007) and this may limit the efficiency of enhancing populations and may thus not increase fish production and catches (Svåsand *et al*., 2000; Araki & Schmid, 2010).

High mortality rates of released captive-reared fish are thought to be a result of the pronounced differences between the traditional hatchery environment and the natural habitat (Olla *et al*., 1998). Hatcheries provide a homogeneous environment typically lacking structure where predators are absent and food is abundant. Earlier works suggested that conventional rearing does not provide satisfactory stimuli for the fish to develop skills associated with survival and that hatcheries might generate behavioural deficiencies and traits disadvantageous for survival in the wild (Olla *et al*., 1998; Salvanes & Braithwaite, 2006). One specific concern is the lack of suitable antipredator behaviours among hatchery-reared fish compared with wild individuals (Berejikian, 1995; Álvarez & Nicieza, 2003; Salvanes, 2017). One may therefore hypothesise that inferior antipredator behaviour of released fish increases predation mortality and that predation is a major cause for the loss of hatchery fish liberated to the wild (Olla *et al*., 1998; Henderson & Lecther, 2003).

The skills needed by fish to detect and avoid predators is partially heritable (Christensen *et al*., 2014), but prior experience also has a large role in shaping antipredator capabilities (Kelley & Magurran, 2003). Hatchery-reared and wild fish have similar reflex responses to threats, but hatchery individuals are seemingly less risk-averse (Salvanes, 2017), which could result in mortality in environments where the predation pressure is high.

In fishes, escape from danger depends on swimming speed, which in turn is a function of the body length (Bainbridge, 1958; Wardle, 1975). As a fish is growing, its number of potential predators will usually decrease, both because of the prey's improved escape capabilities (Juanes & Conover, 1994; Christensen, 1996) and because predators become increasingly gape limited and unable to consume larger individuals (Sogard, 1997).

To avoid or survive predator encounters, wild fish tend to favour habitats where shelters from predators are available (Savino & Stein 1982; Tabor & Wurtsbaugh, 1991). In hatcheries, conventional rearing may not provide the fish with suitable stimuli for developing abilities to properly utilise complex habitats such as shelters and refuges, whereas fish reared in enriched, structurally complex rearing tanks have been shown to develop a more diverse behavioural repertoire that may make them able to take advantage of available shelter opportunities (Salvanes et al., 2007). Enrichment is defined by Näslund & Johnsson (2016) as "a deliberate increase in environmental complexity with the aim to reduce maladaptive and aberrant traits in fish reared in otherwise stimuli-deprived environments". Among the behaviours reported from experimental works on fish subjected to enrichment, enrichment has been found to potentially increase both learning ability (Strand *et al*., 2010; Salvanes *et al*., 2013) and propensity for sheltering when under threat (D'Anna *et al*., 2012) or in novel environments (Salvanes & Braithwaite, 2005; Näslund *et al*., 2013). It might also improve both context-dependent group behaviour (Salvanes *et al*., 2007), exploratory behaviour (Braithwaite & Salvanes, 2005; Ullah *et al*., 2017), stress recovery (Pounder *et al*. 2016) and swimming ability (Ahlbeck Bergendahl *et al*., 2017). Additionally, works have found that enrichment affects the development of foraging behaviour (Brown *et al*., 2003; Moberg *et al*., 2011; Rodewald *et al*., 2011) and reduces swimming activity (Salvanes & Braithwaite, 2005; Moberg *et al*., 2011). Many of these behaviours could be important for a fish to avoid predators and survive in the wild. The enriched rearing environment could therefore potentially be used to improve post-release survival of hatchery-reared fish.

Most experiments investigating the interaction between the nursery environment and behaviour are laboratory experiments, with less input from field experiments to evaluate survival of enriched fish after release. These field experiments have shown mixed results varying from negative, to lack of, to positive effects of enriched rearing on fish survival and survival-related behaviours (Berejikian *et al*., 1999, 2000; Brockmark *et al*., 2007; Tatara *et al*., 2008, 2009; Fast *et al*., 2008; Hyvärinen & Rodewald, 2013; Roberts *et al*., 2014). These contradictory findings might be due to species-dependent responses to enrichment, but also the type and quantity of enrichment seems to affect behavioural development (Näslund & Johnsson, 2016).

At present, there is limited knowledge about whether simple enrichment during rearing in realistic, high-density hatchery conditions in combination with standard release procedures, improve survival after release. Here we present results from such an experiment conducted to test if in-water structural enrichment (shelter) can promote predator avoidance and long-term survival of fish. To investigate this, we released group-marked hatchery-reared age 0+ year Atlantic salmon *Salmo salar* L. 1758 fry from enriched and control treatments into natural streams. In the first part of this experiment, we compared the short-term post-release predation mortality and size-selective feeding by piscine predators on released fry from enriched and control treatments. This was done by searching for released fry in the stomach contents of predators (primarily brown trout *Salmo trutta* L. 1758) resident at the release site.

The predators were sampled 4 h and 48 h after the release of the fry. We expected to find fewer enriched fry compared with controls in the sampled predator stomachs, as previous laboratory experiments have shown more risk-averse behaviour in enriched fish (Salvanes & Braithwaite, 2005; D'Anna *et al*., 2012; Näslund *et al*., 2013). We also expected the predators to feed more on the small fry than on larger ones, because large prey are more difficult to catch and handle (Juanes & Conover, 1994; Christensen, 1996). Gape-size limitation often leads to predators selecting unequal proportions of small and large individuals. This is especially true for piscine predators that commonly select smaller individuals for maximal capture success (Sogard, 1997). In the second part of the experiment, we compared the survival of enriched fry and control fry, months after release, by comparing recaptures from experimental fishing. We expected enriched individuals to be recaptured at a higher rate as a result of their potentially more diverse behaviour repertoire (Braithwaite & Salvanes, 2005; Salvanes *et al*., 2007) which could benefit their foraging abilities (Rodewald et al., 2011) and their suite of behaviour adapted to use shelter (Salvanes & Braithwaite, 2005; D'Anna *et al*., 2012; Näslund *et al*., 2013).

2 | MATERIALS AND METHODS

All procedures have been completed according to the Norwegian Food Safety Authority in compliance with "The Regulation on the use of animals in research" with FOTS id 8706.

2.1 | Experimental fish

The present study was carried out during 2015-2017 using *S. salar* offspring from a captive brood stock, originating from the original Vosso *S. salar* population, housed at Haukvik, which is a part of the Norwegian gene bank programme for *S. salar*. All fish were group-marked in the otoliths at the eyed egg stage using Alizarin Red-S (ARS) at a concentration of 200 mg l^{-1} (Baer & Rosch, 2008), following standard procedures and recommendations by the Norwegian Veterinary Institute (Moen *et al*., 2011). Eggs were separated in two batches on arrival at Voss hatchery, where the rearing took place. Half of the fish were designated for enriched rearing (hereafter referred to as enriched) and were treated with a second alizarin marking, while the other half were reared in a conventional, standard hatchery tank (hereafter referred to as control). The second group marking of the enriched group was not intrusive and should not have had any effect on their growth compared with controls (Baer & Rosch, 2008). The marking resulted in one alizarin mark in the otoliths of control fry and two alizarin marks in the otoliths of enriched fry.

2.2 | Environmental enrichment

Fish were reared in two separate, but similar, rearing tanks (2 × 2 m; each *c*. 2300 l) receiving natural river water from the Vosso River. Structural enrichment was introduced to the tank housing double-ring-alizarin-marked fry at the onset of feeding (*c.* 1–2 weeks after transition to the rearing tank; Table 1). The enrichment consisted of four plastic tube constructions and one green box to provide shelter, both with nylon ropes and plastic shreds attached, to simulate river flora (Figure 1a,b). These structures were cleaned when required, which was *c*. every other week during rearing in June and *c*. every week during rearing in July and August. The enrichment structures were put back to the same place in the tank after cleaning. Both treatment groups of fry were fed under continuous light from above, with commercial pellets (Nutra XP, Skretting; www.skretting.com) dispensed at the water surface by an automatic feeder, five times an hour.

In 2016 the introduction of enrichment had to be delayed (*c*. 2 weeks) due to an outbreak of a fungus infection (*Pseudomonas* sp.) in the rearing tanks. The procedures in 2017 were adjusted accordingly and this resulted in slight variations among experimental years with respect to the duration of rearing and release date (Table 1). The number of fish in the production tanks was reduced once in 2016 (13 July) and twice in 2017 (27 June and 21 July) due to space limitations in the tanks and because the rearing period was longer these years.

2.3 | Stocking of fry

The present study was conducted during three field seasons: 2015, 2016 and 2017 and stocking took place in a stretch of Rasdalselva in Rasdalen and in Teigdalselva in Brekkhus, both tributaries of the Vosso River system. (Figure 2). Hereafter these two release sites will be referred to by their locality names: Rasdalen and Brekkhus, respectively. In 2015 and 2016 stocking was done in Rasdalen only, while in 2017, fish were stocked in both locations. For both release sites, fry were released in small groups and distributed among the substrate along each side of the river.

The release stretch in Rasdalen (release area *c.* 1230 m^2) had a mean width of 10 m (minimum width *c.* = 5 m, maximum width *c.* = 15 m), whereas the release stretch in Brekkhus (release area *c.* 2300 m^2), had a mean width of 21 m (minimum width *c.* = 19 m, maximum width *c.* = 22 m). Both locations consisted of riffles, runs and pools and substrate mainly consisting of larger stones and small boulders, although the Rasdalen location had more pools and somewhat slower water velocity compared with Brekkhus. However, both locations encompass habitat conditions generally considered suitable for rearing *S. salar* juveniles. Both release sites were located above a migration obstacle preventing the wild population to reach this area and thus had no natural production of *S. salar*. However, at both release sites there were natural populations of resident *S. trutta*. Furthermore, both areas had in preceding years (2013 and 2014) been used for stocking of *S. salar* eggs and fry, resulting in presence of some older year classes in the release stretches.

To obtain the size composition of fry in control and enriched rearing tanks, we measured a random subsample of *c.* 100 individuals before collecting fish to be released (Table 2). The fish were transported in transparent 30 l plastic bags filled with 1/3 water (10 l) and 2/3 oxygen from an oxygen tank. Every bag contained an even mix of enriched and control fry, with a total weight of *c.* 1 kg per bag. A total amount of 3600 individuals (1800 from each treatment) were brought to the release site each experiment year. On arrival at the release site, the fry were first transferred to 10 l containers with a mix of water from the transport bag and water from the river (to reduce temperature difference between river and hatchery) before they were released shortly thereafter.

2.4 | Post-release predator sampling procedure

Larger resident salmonids considered as potential predators of the fry (standard length, L_S > 100 mm), were sampled 4 h and 48 h after release of fry. They were sampled using point electrofishing with battery powered backpack generators with a pulsed current of 1400 V and a range of maximum 1 m. To collect the stunned predators, the electrofishing team used hand nets and transferred the fish to containers of river water before they were taken ashore for examination.

The entire length (and some additional meters downstream) of the experimental release stretch were fished by two people for approximately 30–60 min until the entire stretch had been covered. The potential predators were identified to species and anaesthetised with MS-222 to enable L_S measurements (to the nearest mm) and evacuation of stomach contents in order to collect the salmon fry consumed.

2.5 | Stomach content examination

Predator stomachs were examined using a gastric lavage technique (Bromley, 1994). Stomach contents were flushed out with water using a 60 ml syringe fitted with a thin aquarium tube (diameter: outer, 9.0 mm; inner, 0.6 mm), inserted into the mouth of the fish to the distal parts of the stomach. The flushing lasted for *c* 2 min (depending on the amount of fry the predator had consumed) and stomach contents were flushed onto a sieve to remove excess water, before it was put in a cooler to slow the decomposition process and later frozen. The predators recovered from anaesthesia in a 30 l tank containing river water, before they were released back into the river. In 2016 we had permission to lethally sample predators and all predators were euthanised by an overdose of MS-222 before they were put in a cooler and then frozen for later examination of their stomach contents. The same procedure was followed for some predators in 2017 (n = 23) to avoid damaging predators that seemingly had consumed fry, but for which the flushing was unsuccessful. Five of the euthanised predators in 2017 had consumed released fry.

2.6 | Recapture of fry from the river

Between 2–3 months after the release of fry we returned to the release sites to electrofish subsamples of fry and to identify the proportions of control and enriched fry remaining in the river (Table 1). The sampling procedure using point electrofishing was the same as for sampling predators just after fry releases, but now we included another 50 m downstream to sample fry that had dispersed downstream. Recaptured fry were euthanised using an overdose of MS-222. The sampling lasted until about 100 fry released 2–3 months earlier were caught.

2.7 | Measuring fry and examining otoliths

Fry sampled from production tanks, fry consumed by predators and fry recaptured from the river 2–3 months after release were measured; L_S to the nearest mm. For digested fry, it could sometimes be difficult to evaluate what was the end of the vertebral column and hypural bones. We did this to the best of our ability and used a scoring system (Table 3) where each fish was scored for the potential influence of digestive state on length measurement. For analysis, only lengths of fish that scored 0 were used. This led to 29 of the treatmen*t*-identified fry from predator stomach contents to be removed from further analysis of length (2016, $n_{enriched}$ = 8, $n_{control}$ = 14; 2017, Rasdalen, $n_{enriched}$ = 1, $n_{control}$ = 5; Brekkhus, $n_{enriched}$ = 1, $n_{control}$ = 0). Note that this scoring system was first developed after 2015 and thus only used for the data from 2016 and 2017.

For fry sampled from the production tanks and fry recaptured from the rivers, additional measures of wet mass (M_W, to the nearest 0.01 g) was conducted and Fulton's condition factor (K)was calculated (Fulton, 1904; Bolger & Connolly, 1989): $K = 100 M_W L_S^{-3}$, where L_S is the standard length of the fish in millimetres (mm) and M_W is the wet mass of the fish in grams (g).

Fry consumed by predators and fry recaptured from the river 2–3 months after release were assigned to the enriched or the control group based on inspection of otoliths for alizarin marks. The sagittae otoliths were extracted and fixed on individual slides using temporary mounting wax (CrystalBond; www.aremco.com, or QuickStick; www.innovatekmed.com) before they were polished with grinding paper until the daily increments of otoliths were visible (Wright *et al*., 2002). Next, the number of fluorescent rings were identified using an epifluorescent microscope (Zeiss Axioscope 2 plus; www.zeiss.com) and UV-light. Of the fry consumed by predators, 410 individuals could be identified to rearing treatment, but 10 (2.4 %) were unclear and therefore remained unknown. Of the fry recaptured from the river, 440 individuals could be identified to rearing treatment, while 19 (4.3 %) were unclear and remained unknown.

2.8 | Statistical analysis

All statistical analyses were carried out using R version 3.4.4 (www.r-project.org) and the additional libraries Rmisc (Hope, 2013), plyr (Wickham, 2011) and ggplot2 (Wickham, 2016). If fry consumed by predators or recaptured 2–3 months later were either unknown rearing or age ≥ 1 year, they were excluded from all analysis.

Effects of rearing treatment on size (L_S and M_W) and condition at the release date (all years) and at the recapture date (only for 2015 and 2016) were tested using a two-sample *t*-test. For 2017, when fry were released on two sites, the test on recaptured data was done using a two-way ANOVA. Release site (Brekkhus and Rasdalen) and rearing treatment (enriched and control) were specified as categorical predictors and the interaction term was removed from the model if it was not significant. In addition, we tested for differences in length-frequency distributions between treatment groups, by comparing cumulative relative length-frequency distributions using a two-tailed two sample Kolmogorov-Smirnov test (KS-test).

For predation mortality, we first tested whether the proportion of consumed enriched fry and control fry varied between the time of predator sampling (4 h and 48 h after release) by using a χ^2-test of independence. Next, we used two-tailed a χ^2 goodness of fit test to test if enriched and control fry had been consumed in unequal proportions by predators within 48 h after release. The data from Brekkhus were excluded from this analysis due to a low sample size of consumed fry. The a χ^2-test was also used to test if there were similar proportions of enriched and control fry in the recaptured samples (2–3 months after release) and each year and each sampling site was tested separately.

Size-selective predation was tested by comparing length-frequency distributions between fry on the day of release with those consumed by predators within 48 h using a two-tailed two sample KS-test. Due to a small sample size of consumed fry at Brekkhus, Brekkhus was excluded from the KS-test on size-selective predation.

3 | RESULTS

3.1 | Size after rearing

Sixteen-week old control fry were longer after rearing treatments than enriched fry in 2017 (*t*-test: $t_{191} = 2.32$, $P < 0.05$). The same trend was found in 2016, when the fry were 17 weeks old when rearing treatments were completed (*t*-test: $t_{227} = 1.93$, $P < 0.05$). In 2015, however, when the fry were 12 weeks old on the last day of rearing treatments, control and enriched fry had similar lengths (*t*-test: $t_{186} = 0.70$, $P > 0.05$). Inspections of the cumulative length-frequency distributions for each year separately, showed that for 2015 the distributions were similar (KS-test: $D = 0.07$, $P > 0.05$; Figure 3a), for 2016 the two rearing treatments had significantly different length distributions at release (KS-test: $D = 0.20$, $P < 0.05$; Figure 3b), while 2017 they were similar (KS-test: $D = 0.12$, $P > 0.05$; Figure 3c). Enriched and control fry had similar mass in all the experimental years (*t*-test: 2015, $t_{186} = 1.47$, $P > 0.05$; 2016, $t_{224} = 0.93$, $P > 0.05$; 2017, $t_{204} = 1.26$, $P > 0.05$). Enriched fish, however, had a higher condition factor in 2017 (*t*-test: $t_{169} = 3.84$, $P < 0.001$) with similar trends also appearing in both 2016 and 2015, although not significant (*t*-test: 2015, $t_{186} = 1.70$, $P > 0.05$; 2016, $t_{241} = 1.90$, $P > 0.05$).

3.2 | Predation on released fry

A total of 126 potential predators on released *S. salar* fry (123 resident *S. trutta* and 3 *S. salar* from previous stocking) were caught in the river system of Rasdalen and Brekkhus 4 h and 48 h after release in 2015, 2016 and 2017. Of these, 78 (62%) of the predators had consumed a total of 420 released fry (of which 410 individuals could be identified to rearing treatment). Number of fry consumed by predators varied among years and with the size of fry released (Table 2; Table 4). Few of the potential predators caught at Brekkhus had consumed released fry. The data show that larger predators were more likely to consume released fry (Figure 4).

3.2.1 | Predation on enriched and control fry

For each of the years 2016 and 2017, predators had consumed similar proportions of enriched and control fry at both sampling times (4 h and 48 h) after fry were released (χ^2-test: 2016, $\chi^2 = 0.00$, $P > 0.05$; 2017, $\chi^2 = 1.12$, $P > 0.05$; Table 5). The data from 4 h and 48 h could therefore be pooled. The data from 2016 show that predators had consumed fewer enriched than control fry during the first 48 h after release of fry (χ^2-test: 2016, $\chi^2 = 9.08$, $P < 0.01$). This was not the case for the data from 2015 and 2017 when predators ate similar amounts of enriched and control fry within the first 48 h (χ^2-test: 2015, $\chi^2 = 0.06$, $P > 0.05$; 2017, $\chi^2 = 0.04$, $P > 0.05$).

3.2.2 | Size-selective mortality

In 2015, when fry were released in mid-July *c.* 12 weeks after hatching, there was no size-selective predation mortality of any of the treatment groups (KS-test: enriched, $D = 0.17$, $P > 0.05$; control, $D = 0.13$, $P > 0.05$; Figure 5a,b). In 2016 and 2017, the 2 years when fry were released in mid-August 16–17 weeks after they hatched, the fry consumed were smaller compared with the fry’s size distribution at release for both treatment groups (although only weakly significant for the enriched group in 2017) (KS-test: enriched 2016, $D = 0.\ 22$, $P <$

0.05; control 2016, $D = 0.39$, $P < 0.001$; enriched 2017, $D = 0.24$, $P = 0.047$ 0.05; control 2017, $D = 0.25$, $P < 0.05$; Figure 5c–f).

3.3 | Recapture of stocked fry 2-3 months after release

A total of 459 fry were recaptured 2–3 months after they were released; 440 of these could be identified to rearing treatment. The data from 2017 at Rasdalen show that fewer fry from enriched treatments than control treatments were recaptured from the river (χ^2-test, $\chi^2 = 6.82$, $P < 0.01$; Table 6). In 2015 and 2016 similar numbers of enriched and control fry were recaptured at Rasdalen (χ^2-test: 2015, $\chi^2 = 0.20$, $P > 0.05$; 2016, $\chi^2 = 0.60$, $P > 0.05$), which also was the case in the data from Brekkhus in 2017 (χ^2-test: $\chi^2 = 0.28$, $P > 0.05$).

3.3.1 | Size of recaptured fry

Fry from both treatments, all years, had a longer L_S in the recaptured subsample compared with the subsample taken the day of release. The length range was also in general wider at release compared to at recapture (Figure 6). Size distributions of recaptured enriched and control fry were similar for all sampling years (KS-test: 2015, $D = 0.16$, $P > 0.05$; 2016, $D = 0.23$, $P > 0.05$; 2017, Rasdalen, $D = 0.24$, $P > 0.05$; 2017, Brekkhus, $D = 0.07$, $P > 0.05$). There was no difference in L_S, M_W or K between recaptured enriched and control fry from Rasdalen in 2015 (t-test: L_S, $t_{126} = 0.93$, $P > 0.05$; M_W, $t_{125} = 0.52$, $P > 0.05$; K, $t_{122} = 1.55$, $P > 0.05$) or in 2016 (t-test: L_S, $t_{89} = 1.17$, $P > 0.05$; M_W, $t_{91} = 1.10$, $P > 0.05$; K, $t_{103} = 0.01$, $P > 0.05$). There was a difference in 2017 (ANOVA interaction release site * treatment: L_S, $F_{1,201} = 4.62$, $P < 0.05$; M_W, $F_{1,201} = 5.37$, $P < 0.05$) where recaptured control fry were longer and weighed more than enriched fry at Rasdalen (L_S, $P < 0.01$, M_W, $P < 0.01$; Table 7), while at Brekkhus enriched and control were of similar size (L_S, $P > 0.05$, M_W: $P > 0.05$; Table 7). There was no interaction in condition factor between release site and treatment (ANOVA: $F_{1,201} = 0.14$, $P > 0.05$) and the interaction term was therefore removed from the model. The condition of fry recaptured at Brekkhus was higher than of fry recaptured at Rasdalen (ANOVA: $F_{1,202} = 53.99$, $P < 0.001$; Table 7). Treatment had no effect on condition in 2017 (ANOVA: $F_{1,202} = 2.44$, $P > 0.05$).

4 | DISCUSSION

This study has investigated the survival of hatchery-reared *S. salar* fry from an enriched and a conventional rearing treatment, both reared at high fish densities commonly used in restocking programmes. Scrutinising alizarin-marked otoliths allowed us to identify fry from enriched *v.* control treatments both from predator stomachs (even when several fry had become partially digested) and from fry samples recaptured from the rivers. This is the first time, that we know of, that immediate post-release predation mortality of two differing rearing treatments has been evaluated.

Whether or not enrichment during rearing promotes fry survival after release can depend on many factors. River conditions at the release site such as water temperature, number of predators, available shelters and available food items for predator and prey, are likely to change annually and can affect both predator and fry behaviour. The density of released fry and their individual size at release can influence competition between fry and the number of fish available for the predators. It is well known that high fish densities increase competition for limited resources (space and food; Kalleberg, 1958) and that large individuals tend to dominate smaller fish (Metcalfe *et al*., 1989; Adams *et al*., 1998). Also, if a gentle transfer between the hatchery and river is not achieved, it can provoke a stress response in the released fish (Brown & Day, 2002). Furthermore, to be able to detect differences in survival of released fry, it is also required that the sample size is sufficiently large. Our study of predation mortality relies on a sufficient sample size of predators that had consumed fry. Our field data showed inconsistent results between the three experimental years and provided limited support for the hypothesis that enrichment can improve post-release survival of hatchery-reared fish. The data showed that: (1) predators took small prey in two out of three sampling periods; (2) enrichment did not consistently reduce immediate predation mortality; (3) enrichment did not improve recapture rates 2–3 months after release. Only one of the year's predation mortality findings supported the conclusions from previous experimental works, suggesting that the hypothesis that enriched rearing can produce fish with a beneficial risk-averse behaviour is not always true. Our data on recaptures either consisted of similar numbers of control and enriched fry, which occurred in three out of four samples and are similar results to those of Brockmark *et al*. (2007) and Tatara *et al*. (2009), or they comprised of a larger amount of control fry (one site on one date), which was similar to the finding by Berejikian (1999).

4.1 | Immediate predation mortality

The primary predator in this experiment, *S. trutta*, is a facultative piscivore and can often switch to a piscivorous diet if the individual predator is large enough and there is a sufficient density of suitable prey fish available (Keeley & Grant, 2001; Jensen *et al*., 2008). Like most salmonids, *S. trutta* is primarily a visual predator (Ahlbert, 1976; Mazur & Beauchamp, 2003) and for prey fish, this means that it will be beneficial to have developed suitable predator avoidance behaviours such as being able to locate and utilise shelters (Olla *et al*., 1998). Experimental works have shown that rearing with in-tank shelter can promote spatial learning in *S. salar* (Salvanes *et al*., 2013) and increase their sheltering behaviour when released into a novel environment (Näslund *et al*., 2013). Wild *S. salar* fry utilise interstitial spaces in the river substrate to hide from threats like predators (Gibson, 1966) and by having previous experience from use of shelters, we expected that enriched fish would have an improved ability to find these interstitial spaces compared with control fish during the first two days after release.

In our experiment it seemed that enrichment did not consistently improve the fry's ability to avoid predators. It might be that there was an effect, but its effect was so small that it would require larger sample sizes than we had available in our study to identify it. Our largest sample size of consumed fry was obtained in 2016 in the stomachs of *S. trutta*. The year 2016 was also when predators had consumed more control than enriched fry at the release site and when the stomach-content data available consisted of 233 treatment-identified fry. The sample size of consumed prey in 2016 was 2.3 larger than in 2017 (sample size: 101) and 3.3 times larger than in 2015 (sample size: 71).

The fry in our experiment were released in high densities which is required to create competition between individuals according to Kalleberg (1958). Based on the approximate area of the release sites, the density in the release stretch right after release in Rasdalen was about 290 fish 100 m^{-2} and in Brekkhus it was 160 fish 100 m^{-2}, which are both considerably higher compared with natural densities in other parts of the Vosso River (on average 10–40 fish 100 m^{-2} of age 0+ year *S. salar*; Barlaup, 2017). It could be that under such high-density releases, some fry, regardless of rearing treatment, will struggle to find shelter during a predator threat, due to competition over the limited number of shelters available (Finstad *et al.*, 2007). It might also be that fry end up spending more time competing over shelters instead of actually sheltering (Näslund *et al*., 2013). When salmonids compete for spatial structures, the individuals tend to be more pelagic until a site becomes available (Kalleberg, 1958) and this could make them more prone to predation. Enrichment could potentially improve the competitive ability of salmonids (Berejikian *et al*., 2000; 2001), but we do not know if this is true for our experiment.

A factor that could have reduced the fry's acquired antipredator behaviour is stress induced by the release procedure (Olla & Davis, 1989; Olla *et al*., 1995). Stress can also affect other behavioural traits like swimming performance, aggression and orientation negatively (Schreck *et al*., 1997). Depending on the trait and the intensity and duration of the stressor, it could take hours, days or weeks before normal behaviour is recovered (Olla & Davis, 1989; Olla *et al*., 1995; Schreck *et al*., 1997). Enrichment can supposedly reduce time needed for recovery after stress in laboratory experiments (Pounder *et al*., 2016), but it is not known if this is also true in the wild. Furthermore, we do not know to what extent stress at release could have masked potential effects of enrichment in our experiment.

Size is also an important trait determining survival of juvenile fishes (Sogard, 1997). We found that enriched fry tended to have a slower growth than controls during pre-release rearing in the hatchery. This is in accordance with at least one previous experiment on *S. salar* in enriched environments (Rosengren *et al*., 2017), but in contrast to Brockmark *et al*. 's (2007) finding of no difference between the size of enriched and control individuals reared at high densities. Rosengren *et al*. (2017) hypothesised that growth differences could be a result of a preference for hiding instead of feeding if shelters are available. Because we found *S. trutta* predators to feed mostly on smaller prey, there is a possibility that enriched fish could have been more prone to size-selective feeding by predators, since the size distribution of fry from enriched rearing seemed to include larger proportions of small individuals than the control-reared fry.

4.2 | Size-selective mortality

Size-selective mortality caused by predation was documented in two out of three experimental years (2016 and 2017), which were the years when fry were released in August. These years *S. trutta* fed mostly on smaller fry. No size-selective predation mortality was detected in the data from 2015. In this year, the fry were released one month earlier (July) and were thus on average much smaller (34 mm) than in 2016 (*c.* 50 mm) and 2017 (*c.* 56 mm). Moreover, maximum lengths of consumed fry relative to their predator were 25%, 36 % and 46% of predator's length for 2015, 2016 and 2017 respectively.

Our data on size-selective mortality suggest that prey size is important for whether a predator pursues and consumes a prey, or more small-sized fry were available for predators. The former is supported by the relationship between escape capabilities and size of prey, where the ability to escape predator attacks often will improve with growth (Christensen, 1997), while the latter could be a result of behavioural differences between small and large fry (Metcalfe *et al*., 1989). For size-selective mortality to occur there must be a size variation among individuals (Sogard, 1997) and in this study this variation was largest for the 2 years where size-selective mortality was detected. For these 2 years, the larger 10% of the subsample collected the last day of rearing was 1.6 times and 1.7 times larger than the smaller 10% of the subsample for 2016 and 2017, respectively. It is probable that the larger fry would have had an advantage when it comes to escaping predator attacks because of their theoretically higher burst swimming speed (Wardle, 1975). This, together with potential difficulties for predators in handling and manipulating larger fry (Juanes & Conover 1994), are likely contributors to the size-selective predation mortality on small fry in our experiments. Piscivorous *S. trutta* commonly select smaller individuals if given the opportunity to do so (Jensen *et al*., 2008), which leads to a decreasing number of potential predators on a prey as the prey increases in size.

In addition to the predator's selection of small prey, a prey's behaviour will also affect its predation risk. Large individuals of salmonids are more aggressive than smaller fish and in contests over limited resources such as space and food, the largest often win the contest (Metcalfe, *et al*., 1989; Adams *et al*., 1998). Individual juvenile salmonids that search for vacant space are usually more pelagic until they have found suitable sites (Kalleberg, 1958). It is likely that these patterns also occur among released fry. This might restrict the ability of smaller released fry to find and keep sheltered positions and thus make them more available as prey for predators. If so, this will strengthen to the negative size-selective mortality.

Our findings are consistent with the results of earlier works finding that if there is a variation of sizes among individuals, piscine predators commonly consume smaller individuals (Sogard, 1997) and both characters of the prey and predators could explain size-selective predation on released fry.

4.3 | Survival after 2-3 months

To survive in the river habitat, released fry must not only be successful avoiding predators, but they must also learn to forage on new food items and to defend quality territories from competitors. Enrichment has in earlier experiments been shown to improve fishes' learning ability (Strand *et al*., 2010; Salvanes *et al.*, 2013) and behavioural flexibility (Braithwaite & Salvanes, 2005; Salvanes *et al*., 2007), which have been considered to be valuable skills to have in an ever-changing habitat like a stream. However, in our experiment it seemed that enrichment did not improve long-term survival compared with control fry, as estimated by recaptures in subsamples collected 2–3 months after release. Interestingly, for one of the years, a higher proportion of controls were caught, suggesting a possible negative effect of enrichment. Negative effects could have been the tendency of enrichment to reduce growth, or it might also be that the risk-averse behaviour that has been previously documented in fry reared with shelters (Salvanes & Braithwaite, 2005; *D*'Anna *et al*., 2012; Näslund *et al*., 2013) could actually limit the enriched fry's foraging and further survival after release. Experiments conducted by others have shown mixed results with respect to the effect on enrichment on the survival of salmonids and field experiments have reported both positive effects (Hyvärinen & Rodewald, 2011); lack of effects (Brockmark *et al*., 2007; Tatara *et al*., 2009); and negative effects (Berejikian *et al*., 1999) on survival. Although these experiments differ in species studied, salmonid life stage tested, quantity, type and timing of enrichment provided during rearing and sampling procedure, they show, together with our data reported here, that the benefits from enrichment on post-release survival are not straight forward.

Enriched fish might have steeper learning curves early in the encounter with novel areas (Salvanes *et al*., 2013), but controls will also gain experience with time (Salvanes *et al*., 2013, figures 3 and 4). Also, several previous works have documented how fish surviving their first predator encounter are better at surviving new ones (Olla & Davis, 1989; Berejikian, 1995) and an experiment investigating foraging ability in enriched and control *S. salar* parr found that the initial difference (in favour of enriched fish) were weakened after some weeks (Rodewald *et al*., 2011).

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The initial size differences between treatment groups, unlike experience, does not necessarily change with time. Differences can be maintained or increased through size-selective predation, by forcing smaller individuals to live in areas where the trade-off between shelter from predators and feeding opportunities are less fortunate (Werner *et al*., 1983). In our experiment, the initial tendency towards a difference in length between enriched fry and control fry on the day of release seemed to have been maintained at least at

Rasdalen for 2–3 months for all years. Interestingly, the year with the largest mean length difference between enriched fry and controls was also the year with a higher proportion of controls recaptured at Rasdalen 2 months later. Perhaps the size of released fish was a more important factor for survival over time, when both rearing treatments obtained experience in the wild. What has been shown by others is that size at release is important for survival and survival is higher if fish are released at a size at which they are less vulnerable to predators (Svåsand *et al*., 2000; Hyvärinen & Vehanen, 2004). Maybe the enrichment treatment could have shown beneficial effects if we had released optimal fish sizes for survival among piscivorous predators? However, one must also keep in mind that keeping fish longer in captivity, may result in potential negative domestication effects and higher costs (Svåsand *et al*., 2000).

Given the many factors that potentially can influence the survival of released hatchery-reared fish and the fact that replications of field experiments are difficult due to potential annual variations, our data cannot provide a categorical conclusion on whether structural enrichment during rearing can improve survival after release. It seems that structural enrichment alone is not able to improve the survival of *S. salar* fry, especially not in high-predation areas. It is not unlikely that the effects of certain types of enrichment can vary with the life stage and species of fish (Näslund & Johnsson, 2016). Other and additional practices might be needed for improving post-release survival. For example, earlier experimental works found positive effects of reduced rearing density (Brockmark & Johnsson, 2010; Rosengren *et al*., 2017), predator conditioning (Olla & Davis, 1989; Brown & Laland, 2001), large-scaled acclimatisation–habituation procedures (Brennan *et al*., 2006; Strand & Finstad, 2007, Sparrevohn & Støttrup, 2007), alternative or more enrichment types (Roberts *et al*., 2014), or they stocked fish at a size where they are not as prone to predation (Svåsand *et al*., 2000; Hyvärinen & Vehanen, 2004).

Further large-scale research on the practice of hatchery-rearing and release are required in order to find the most optimal strategy for obtaining higher survival in released hatchery-reared fish. Even though we did not find any consistent benefit of enriched rearing in our experiment, this does not mean that the use of enrichment should be ignored. It could still be used as a tool for improving the development of the fish brain (Salvanes *et al*., 2013), potentially improve fish welfare (Sneddon, 2011) and in the future we might understand what enrichment type in combination with other factors should be used when and for which species, in order to successfully enhance depleted wild populations.

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Contributions

A.G.V.S. had the idea and designed the study and raised the funding; A.G.V.S. and M.R.S. collected the field data and M.R.S. did the lab work and examined fish otoliths under supervision of H.S.; M.R.S. analysed the data under supervision of A.G.V.S. and H.S.; M.R.S. wrote the first version of the manuscript under supervision of A.G.V.S. and H.S. All authors have commented on the text and approved the final version.

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TABLE 1 Information related to the rearing, release and later recapture of *Salmo salar* fry in 2015, 2016 and 2017. The last day of environmental enrichment was the same day as fry were released. Recapture refers to the sampling of *c*. 100 fry from the release site 2–3 months after release

Year	**Hatching date**	**Transition to rearing tank**	**Fish moved to each tank (*n*)**	**Enrichment duration (weeks)**	**Age at release (weeks)**	**Release date**	**Release site**	**Recapture date**	**Recaptured fry (*n*)**
2015	13–19 Apr	27.05	8300	*c*. 5	*c*.12	07 July	Rasdalen	07 Oct	133
2016	18–24Apr	26.05	16,000	*c*. 8	*c*. 17	17 Aug	Rasdalen	24 Oct	111
2017	24–30 Apr	23.05	16,000	*c*. 10	*c*. 16	15 Aug	Rasdalen	08 Nov	122
2017	24–30 Apr	23.05	16,000	*c*. 10	*c*. 16	15 Aug	Brekkhus	08 Nov	93

TABLE 2 Mean (± SD) standard length (L_S), wet mass (M_W) and Fulton's condition factor (*K*) of subsampled *Salmo salar* fry from each production tank at the last day of rearing in 2015, 2016 and 2017

Year	**Treatment**	***n***	**L_S (mm)**	**M_W (g)**	***K***
2015	Enriched	93	34 ± 3	0.73 ± 0.15	1.84 ± 0.18
2015	Control	95	34 ± 3	0.69 ± 0.16	1.80 ± 0.17
2016	Enriched	127	49 ± 8	2.11 ± 0.94	1.67 ± 0.10
2016	Control	123	51 ± 6	2.21 ± 0.65	1.65 ±0.12
2017	Enriched	107	55 ± 9	3.04 ± 1.22	1.76 ± 0.16
2017	Control	128	57 ± 7	3.22 ± 1.00	1.69 ± 0.10

TABLE 3 The scoring system used in 2016 and 2017 to evaluate the influence of the state of digestion on standard length (L_S) measurements of *Salmo salar* in *Salmo trutta* stomachs. This was used to determine the certainty of the length measurements of consumed fry

Score	**Definition**
0	Minimal influence on measurement of L_S
1	Deformations of head or L_{VC} that may influence measurement of L_S
2	Deformations in head and, or L_{VC} deformed that will influence measurement of L_S
3	Substantial part of individual missing; L_S unobtainable.

L_{VC}, vertebral column length.

TABLE 4 Overview of potential predators, *Salmo salar* and *Salmo trutta* sampled 4 and 48 h after release of *S. salar* fry at Rasdalen and Brekkhus in 2015, 2016 and 2017.

Year	**Release date**	**Release site**	**Hours after release**	**Sample size (*n*)**	**Feeding fish (*n*)**	**Total fry consumed* (*n*)**	**Prey per feeding predator (*n*; mean ± SD)**
2015	07 Jul	Rasdalen	48	8	6	74	12 ± 11
2016	17 Aug	Rasdalen	4	13	8	30 (1)	4 ± 3
2016	17 Aug	Rasdalen	48	33	32	206 (2)	6 ± 4
2017	15 Aug	Rasdalen	4	33	15	54 (6)	4 ± 3
2017	15 Aug	Rasdalen	48	20	13	42	3 ± 3
2017	15 Aug	Brekkhus	4	10†	1	1 (1)	1
2017	15 Aug	Brekkhus	48	9	3‡	3	1 ± 0

*The numbers in parentheses are the number of fry that could not be linked to a specific predator because these fry had been regurgitated by the predator at time of capture. Furthermore, these fry were not included in the calculation of number of prey per feeding predator

† Three of the sampled salmonids were *S. salar*.

Sample size = the number of potential predators (standard length > 100 mm) caught; Feeding fish = predators that had consumed one or more released fry.

TABLE 5 Proportion of *Salmo salar* fry from enriched and control treatments consumed by *Salmo trutta* predators sampled 4 h and 48 h after release of fry at Rasdalen in 2015, 2016 and 2017

Year	**Hours after release**	**Number identified**	**Number enriched**	**Proportion enriched**	**Pearson's χ^2**	***P***
2015	48	71	37	0.52	0.06	> 0.05
2016	4	31	12	0.39	1.16	> 0.05
2016	48	202	81	0.40	7.53	< 0.01
2016	Pooled	233	93	0.40	9.08	< 0.01
2017	4	60	26	0.43	0.82	> 0.05
2017	48	41	23	0.56	0.39	> 0.05
2017	Pooled	101	49	0.49	0.04	> 0.05

Table 6. Proportion of *Salmo salar* fry from enriched and control treatments recaptured from Rasdalen and Brekkhus 2-3 months after release of fry in 2015, 2016 and 2017

Year	Release site	Number identified	Number enriched	Proportion enriched	Pearson's χ^2	*P*
2015	Rasdalen	128	61	0.48	0.20	> 0.05
2016	Rasdalen	107	49	0.46	0.60	> 0.05
2017	Rasdalen	115	43	0.37	6.82	< 0.01
2017	Brekkhus	90	42	0.47	0.28	> 0.05

Table 7. Mean (± S.D.) standard length (L_S), wet mass (M_W) and Fulton's condition factor (K) of *Salmo salar* fry in the recaptured subsample from Rasdalen and Brekkhus in 2015, 2016 and 2017

Year	Site	Treatment	Number recaptured	L_S (mm)	M_W (g)	K
2015	Rasdalen	Enriched	61	39 ± 3	0.96 ± 0.23	1.61 ± 0.11
2015	Rasdalen	Control	67	39 ± 3	0.98 ± 0.22	1.58 ± 0.10
2016	Rasdalen	Enriched	49	52 ± 7	2.08 ± 0.70	1.42 ± 0.11
2016	Rasdalen	Control	58	54 ± 5	2.21 ± 0.55	1.42 + 0.11
2017	Rasdalen	Enriched	43	59 ± 6	2.77 ± 0.73	1.30 ± 0.11
2017	Rasdalen	Control	72	62 ± 5	3.17 ± 0.68	1.28 ± 0.09
2017	Brekkhus	Enriched	42	62 ± 5	3.40 ± 0.74	1.42 ± 0.13
2017	Brekkhus	Control	48	62 ± 5	3.32 ± 0.73	1.39 ± 0.12

FIGURE 1 Schematic illustration of the enrichment used in the enriched rearing tanks. (a) Tube construction that consisted of three black plastic tubes with multiple openings on the sides, assembled by threaded rods. Individual tube: length: 43–53 cm; outer diameter: 9 cm. One bouquet of green and grey nylon threads (length: *c*. 30 cm) and one bouquet of grey plastic shreds (length: *c*. 40 cm) were attached to the tube construction. (b) Green box with opening: length: 60 cm; width: 40 cm; height: 18 cm, with assembled bouquet of green nylon threads (length: *c*. 110 cm).

FIGURE 2 Map of the Vosso River system (only showing the tributaries relevant for this experiment) and the location of the two experimental release sites; Rasdalen and Brekkhus.

Typesetter

1 Delete 0″E from long values; change latitude value to 60° 40′ N.

2 Delete compass arrow and N.

FIGURE 3 Cumulative relative standard length (L_S)-frequency distributions of (a) 12 week old *Salmo salar* fry in 2015, (b) 17 week old fry in 2016 and (c) 16 week old fry in 2017. fry from enriched and control production tanks on the last day of rearing. **—**, Fry from enriched treatment; **- - -**, fry from the control treatment.

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1 Change $\mathrm{L_S}$ to L_S.

FIGURE 4 Standard length (L_S)-frequency distributions of potential salmonid predators (*Salmo trutta* and *Salmo salar*), sampled at the release sites within 48 h after the release of fry, that had (a), (b) eaten ≥ 1 released fry or (c), (d) had not eaten any released fry. ▇, Pooled potential predators caught at Rasdalen in 2015, 2016 and 2017; ▇, potential predators caught at Brekkhus.

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1 Change Ls to L_S.

FIGURE 5 Cumulative relative standard length (L_S)-frequency distributions of *Salmo salar* fry from enriched and control treatments consumed by predators at Rasdalen (4 h and 48 h samples pooled) next to the distribution of fry from the respective production tanks the day of release: (a) enriched fry from 2015; (b) control fry from 2015; and (c) enriched fry from 2016; (d) control fry from 2016; (e) enriched fry from 2017; (f) control fry from 2017. **—**, Fry from the production tanks; **- - -**, fry consumed by predators.

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1 Change Ls to L_S.

FIGURE 6. Standard length (L_S) of *Salmo salar* fry at the day of release (Prod. tank) compared with length at recapture of (a) enriched fry from Rasdalen 2015, (b) control fry from Rasdalen 2015, (c) enriched fry from Rasdalen 2016, (d) control fry from Rasdalen 2016, (e) enriched fry from Rasdalen and Brekkhus in 2017 and (f) control fry from Rasdalen and Brekkhus in 2017. The width of each violin plot (shaded) is positively correlated to the probability of an individual having a specific L_S. The boxplot shows the median (**–**), 25th and 75th percentiles (□), 95% CI (|) and outliers of the data (●).

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1 Change Ls to L_S.

Figure 1

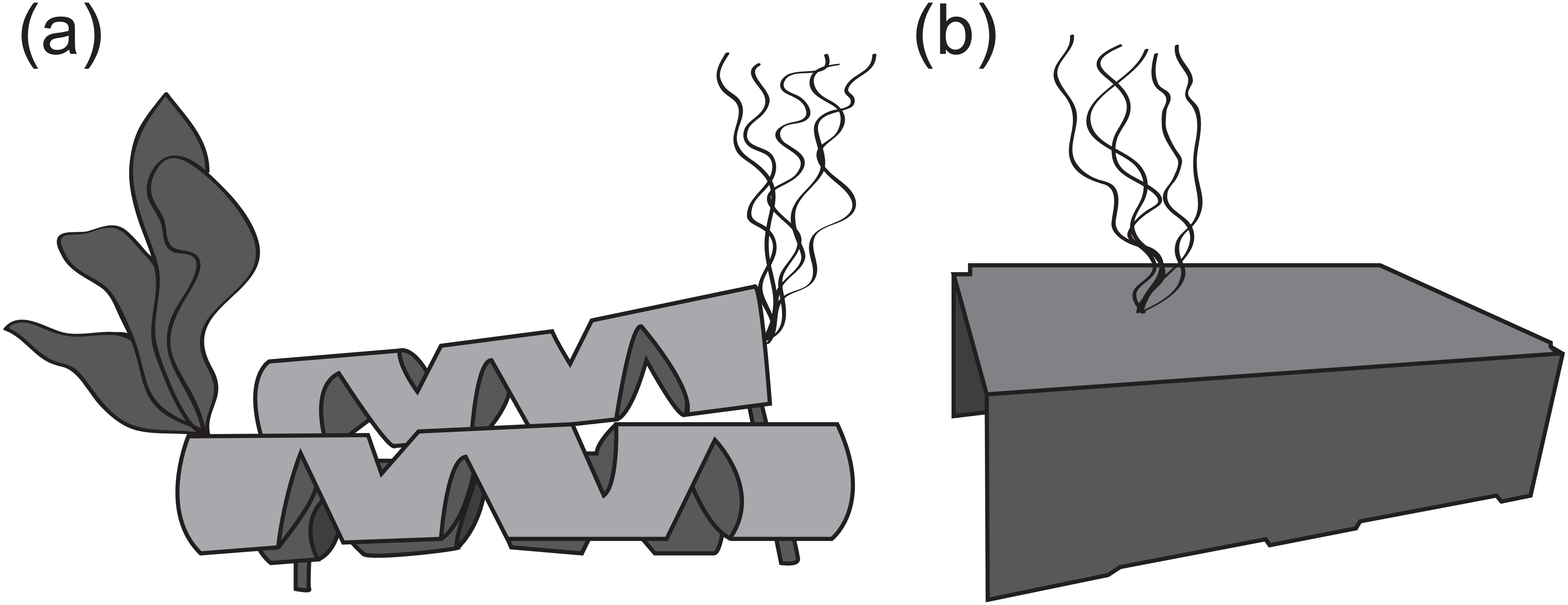


Figure 2

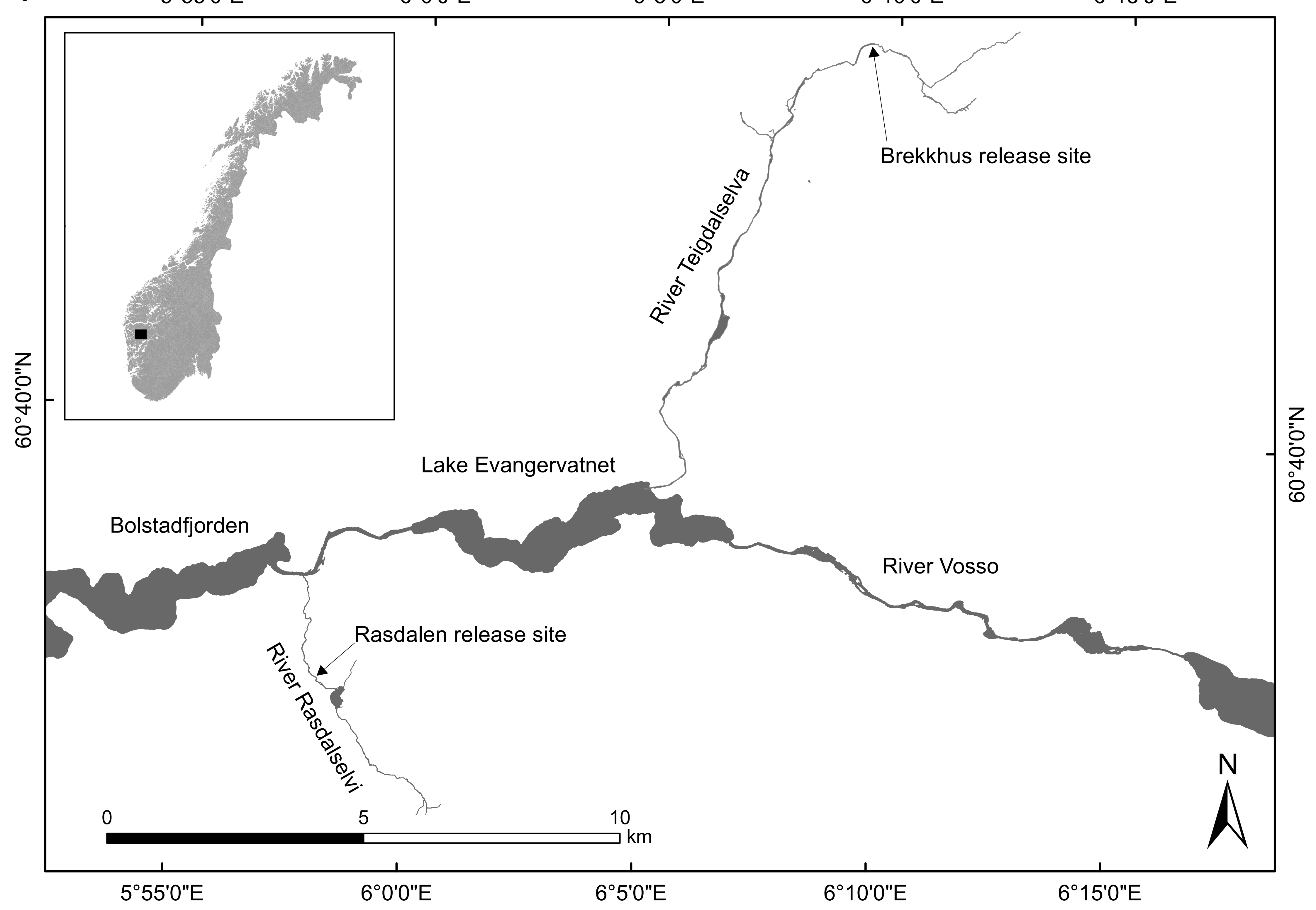

5°55'0"E
6°0'0"E
6°5'0"E
6°10'0"E
6°15'0"E
60°40'0"N
Brekkhus release site
River Teigdalselva
Lake Evangervatnet
Bolstadfjorden
River Vosso
Rasdalen release site
River Rasdalselvi
N
0
5
10
km

Figure 3

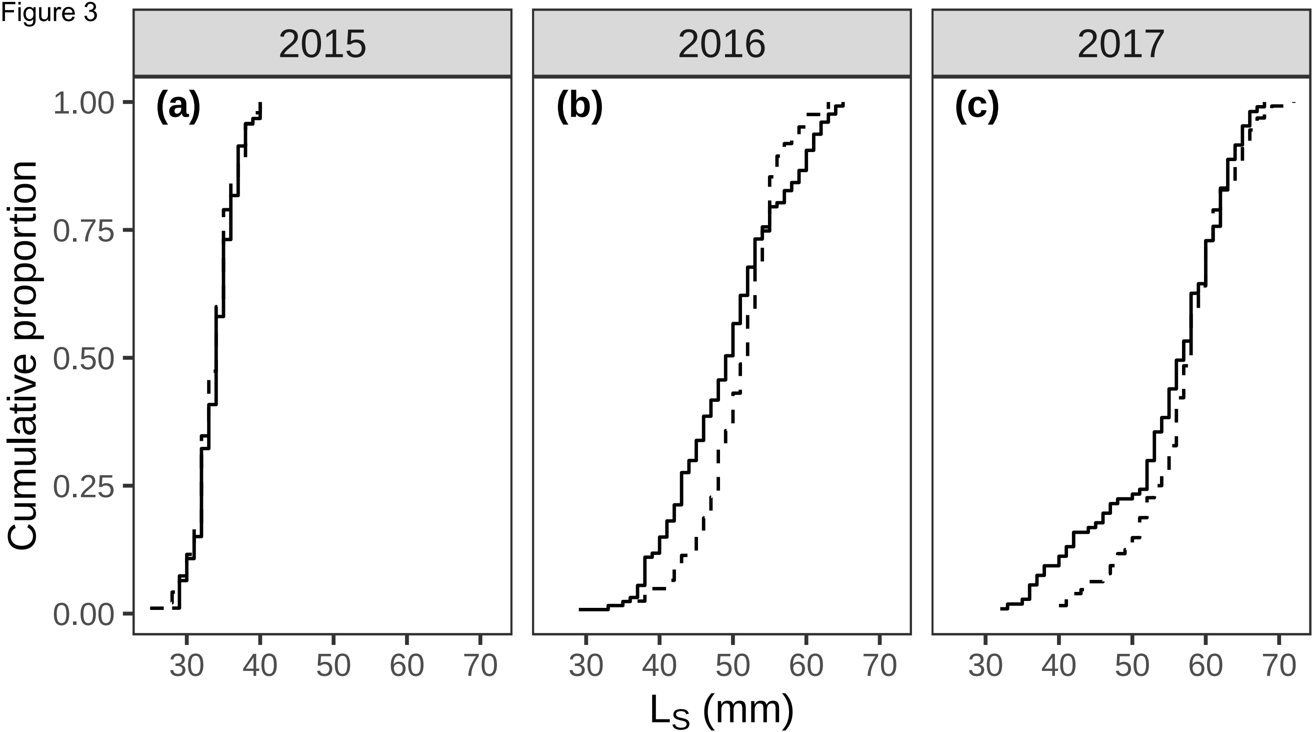

2015
2016
2017
(a)
(b)
(c)
Cumulative proportion
1.00
0.75
0.50
0.25
0.00
30
40
50
60
70
L_S (mm)

Figure 4

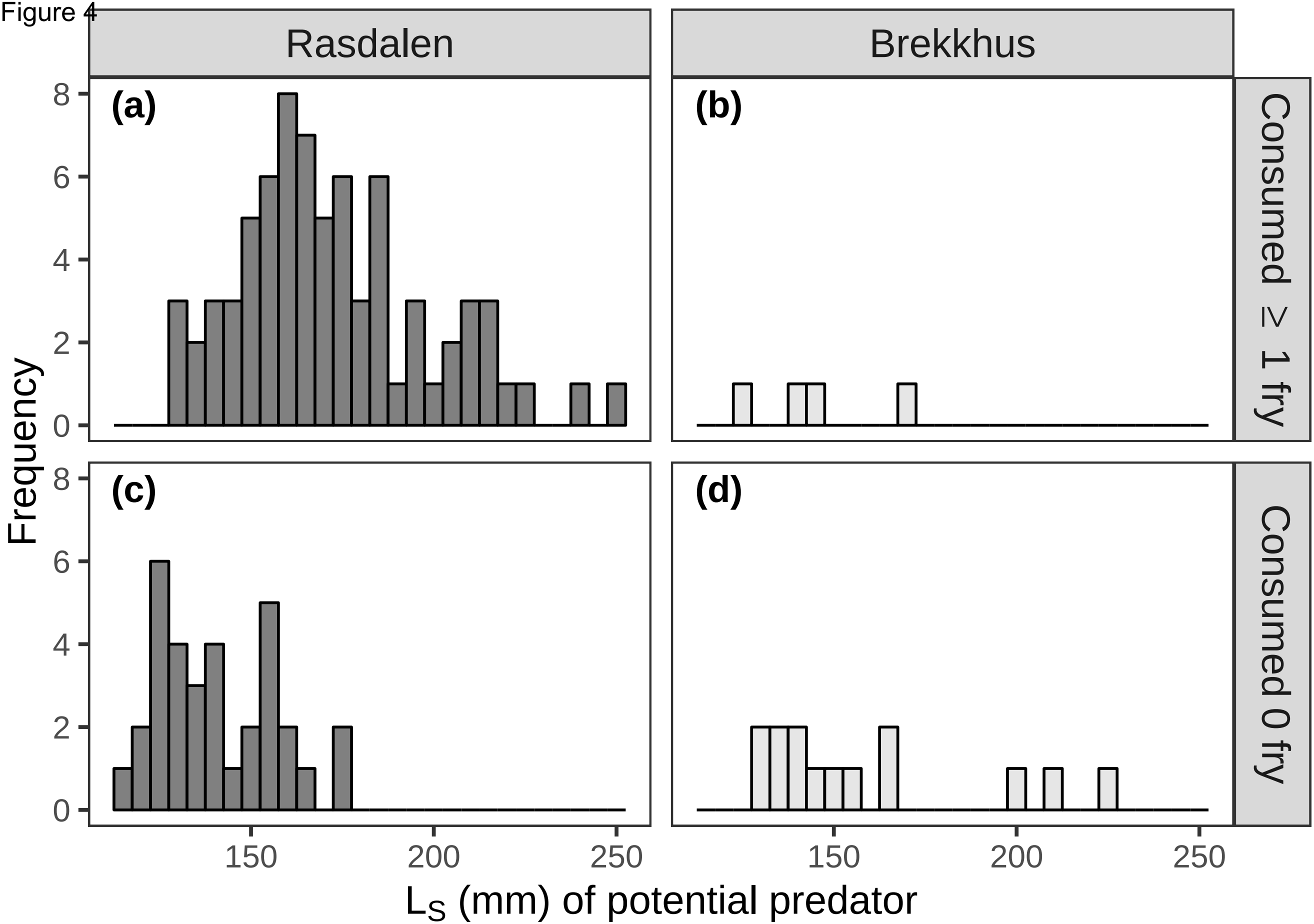
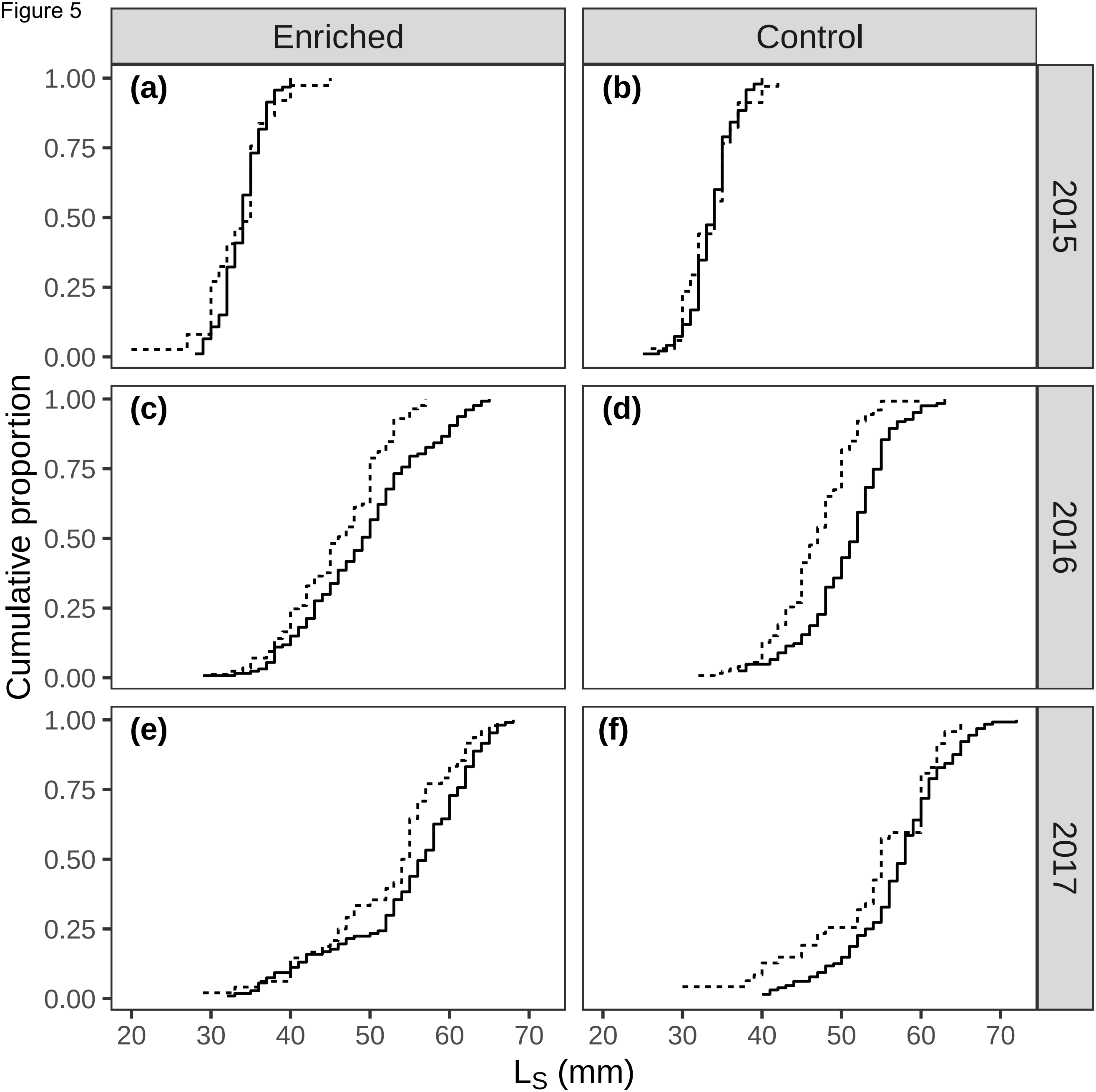
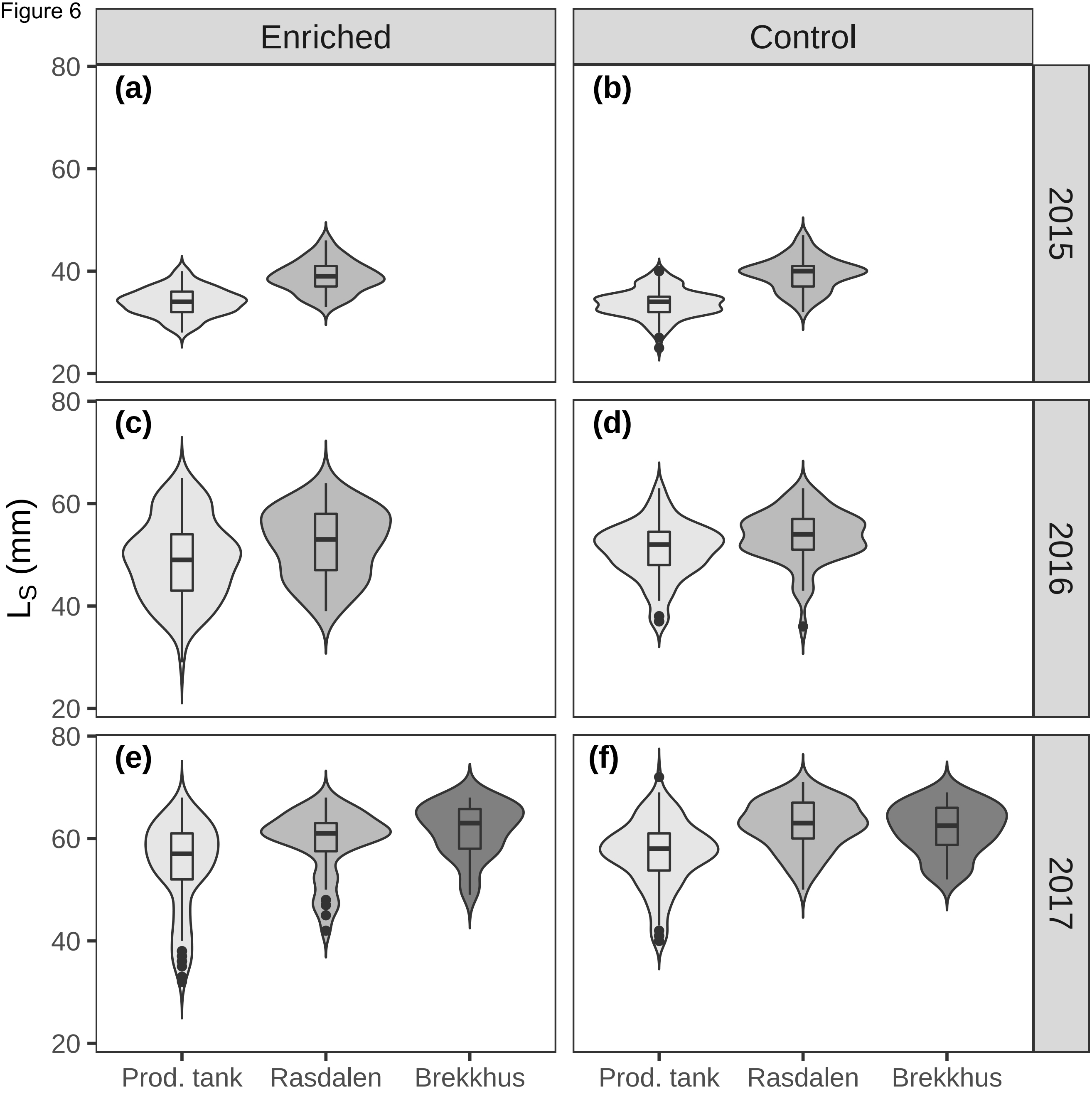

Rasdalen
Brekkhus
(a)
(b)
(c)
(d)
Consumed ≥ 1 fry
Consumed 0 fry
Frequency
0
2
4
6
8
150
200
250
L_S (mm) of potential predator

Figure 5



Enriched
Control
2015
2016
2017
(a)
(b)
(c)
(d)
(e)
(f)
Cumulative proportion
1.00
0.75
0.50
0.25
0.00
20
30
40
50
60
70
Ls (mm)

Figure 6


Enriched
Control
(a)
(b)
(c)
(d)
(e)
(f)
2015
2016
2017
L_S (mm)
80
60
40
20
Prod. tank
Rasdalen
Brekkhus