

BIO-info 05/2012, 3. feb 2012

submission deadline to [bio.info@bio.uib.no](mailto:bio.info@bio.uib.no) is Wednesday 16:00

## Fra toppen!

### Februar

Så er februar måned i gang. Dersom du ikke visste det, kommer navnet februar fra latin *februum*, som betyr *renselse*, og som også er utgangspunkt for ordet *feber*. Selv om mange blir rammet av feber på denne tiden av året, er det visstnok det gamle romerske renselsesritualet som har gitt utgangspunkt for månedens navn.

På kurs- og forelesningssaler er det på denne tiden hektisk aktivitet av studenter og undervisere, og vi satser på å bringe flere inntrykk fra disse aktivitetene i BIO-info fremover. Her håper vi på minst mulig feber, og mest mulig biologisk renselse.

En annen renselsesprosess i disse dager heter Cristin. Her skal fjorårets vitenskapelige produksjon legges inn i en felles database. Fristen er 15. februar, samme dag som det gamle renselsesritualet Februa i den romerske kalender. God vask!

Hilsen Anders



### Ukens bilde



### BIO101

Fotograf: **Beate Rensvik**

Endelig er lab'en startet i BIO101 Organismebiologi 1! Mikrobiologene er først ute og har i god dugnadsånd samlet sammen ansatte, alt fra professorer til stipendiater til å assistere på lab'en..

*You are invited to submit photos (electronically!) for "Ukens bilde". Please include a very short description and credit information. Picture can be of researchers / students in action, technology, organisms, field sites ... Please send your pictures to [bio.info@bio.uib.no](mailto:bio.info@bio.uib.no)*

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# BIO-info

## Nyheter fra Institutt for biologi

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[BIO-info arkiv](#) [Sakslistor & referater](#) [BIOs interne websider](#) [BIO's eksterne websider](#)  
[Facebook BIO](#) [Facebook STIM](#) [Facebook UiB](#)

## VIKTIG INFORMASJON

Big History på Darwindagen;

### Fred Spier foreleser på Darwindagen

MN har satt i gang en ny forelesningsserie som begynner denne våren, med tittel "Horisonter". Det første foredraget i denne serien er **årets darwindag-forelesning, som finner sted mandag 13. februar**. Forelesningsserien "Horisonter" er ment å skulle hjelpe oss alle til å heve blikket, og årets Darwindag-foreleser, **Fred Spier**, konsentrerer seg om de siste 13,7 milliarder år. Mer info på hjemmesiden [www.darwin.uib.no](http://www.darwin.uib.no), og på vdlagte [poster om Darwin dagen](#)

## BIO-arrangement kommende uke

Dato	Handlinger, navn	Tid og sted
6.2.	Disputas Meineri: Assessing and comparing climatic control on distribution and reproduction of alpine and lowland species in the subalpine habitat of western Norway	Mandag 6. Februar kl 12.15, Stort aud., Høyteknologisenteret
9.2.	BIO-seminar Mikko Heino Fisheries-induced evolution: what we know, and what we would like to know?	Torsdag 9 februar 14.15 – 15. K1/K2 A-blokken

## NYHETER OG GENERELL INFORMASJON

[Disputas Meineri](#); [Horizon 2020 er lansert](#); [New subscription list to FunderFinder](#)

### Disputas Eric Meineri

Eric Meineri disputerer for ph.d. graden mandag 6. februar med avhandlingen:

"Assessing and comparing climatic control on distribution and reproduction of alpine and lowland species in the subalpine habitat of western Norway"

Bedømmelseskomite: Senior ecologist Elizabeth Crone, Harvard Forest, USA, Professor Jacob Weiner, Københavns Universitet, Danmark, Professor Peter Emil Kaland, BIO.

Leder av disputasen: Førsteamanuensis John-Arvid Grytnes, Universitetet i Bergen

Tid og sted: Mandag 6. februar 2012, kl. 12.15, Stort auditorium, Høyteknologisenteret, Thormøhlensgate 55

Alle interesserte er velkommen



# BIO-info

## Nyheter fra Institutt for biologi

### Horizon 2020 er lansert

- Europeiske forskere skal bruke mer tid i laboratoriene og mindre tid på å fylle ut papirer, sa EUs kommissær for forskning og innovasjon, Maire Geoghegan-Quinn, da hun lanserte Horizon 2020, som skal avløse det sjuende rammeprogrammet (FP7) i 2014. Kommisjonen foreslår å bruke 80 milliarder euro på programmet som skal løpe over en periode på sju år. **Statsråd Tora Aasland vil foreslå at marin og maritim forskning får en bredere plass i rammeprogrammet.** [Les mer](#)

### New: Subscribe to FunderFinder mailinglist:

The UiB IT department has generated a new FunderFinder mailing list that you can subscribe to. You can sign up by following the instructions here: <http://mailman.uib.no/listinfo/funderfinder>  
Find link to the new issue of FunderFinder under «Nye utlysninger».

## Studie

Masterdag 8. mars

### Masterdag ved BIO 8.mars

Årets masterveke blir arrangert i veke 10 ved Det matematisk-naturvitenskapelige fakultet. BIO sin masterdag er planlagt til torsdag 8. mars, og tidspunktet blir mest sannsynlig 14-16.

Endeleg opplegg og program for masterdagen er under planlegging, men vi vil som i fjor ha behov for at forskningsgruppene stiller opp på dagen. I tillegg vil vi gjerne samle prosjektskiltringar/døme på masterprosjekt frå dei ulike gruppene. Mange av gruppene har no eigne nettsider som viser masterprosjekt, og vi vonar desse blir oppdaterte til masterdagen. Skiltringar av moglege masterprosjekt vil vi og gjerne ha på e-post til [studie@bio.uib.no](mailto:studie@bio.uib.no).

Dersom nokon har gode idear eller har lyst å bidra med foredrag eller anna profilering av mastertilbodet tek Studieseksjonen gjerne i mot innspel og forslag!

## Nyansatte

Vi ønsker følgende nyansatte velkommen til BIO:

Navn/Stilling	Ansatt dato	Forskningsgruppe
Rakesh Madhusoodhanan/Forsker	01.01.2012	Marin mikrobiologi
Liv Sandlund/stipendiat	01.02.2012	Fiskesykdomsgruppen/SFI
Anna Zofia Komicarczuk/postdoc	01.02.2012	Fiskesykdomsgruppen/SFI

## NYE UTLYSNINGER

Mer info om utlysninger inkl. løpende, dvs. uten frister finner du [her](#)

Husk å sende søknadsutkastet til [post@bio.uib.no](mailto:post@bio.uib.no) 1 uke i forveien (gjelder ikke mindre bevilgninger som legater og fonds)

[FunderFinder](#); [Biotek2021](#); [Workshop Early Stage Scientists](#); [Summer School undergraduate and Masters students](#); [Summer Courses Svalbard](#);

### New issue of FunderFinder:

Read the [new issue of UiBs internal magazine FunderFinder!](#) In addition to information on **EU calls** you can find information on how to apply for **EU-project establishing support (PES) for UiB**

# BIO-info

## Nyheter fra Institutt for biologi

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**researchers** and get information on upcoming meetings and courses and about a **new call in ERA NET Martec** and more.

### **Biotek 2021 er i gang**

Programmet [Biotek 2021](#) er i gang og den første utlysningen planlegges med søknadsfrist 30. mai.

Det skal være en obligatorisk skisseprosess med frist 15. mars 2012.

Det meldes at det legges opp til store samarbeidsprosjekter med flere partnere, og med ramme på 3-5 år med bevilgning 10 mill. kr pr år. I denne runden forventes tildeling til ca. fire prosjekter.

Forskningsrådet har lagt opp til en orienteringsrunde om programmet og forventninger til søknadene.

### **Informasjonsmøte i Bergen:**

Tid: Onsdag 15. februar 2012 kl. 11-14.

Sted: Stort auditorium, Datablokken HIB.

Agenda:

- Innledning med informasjon om BIOTEK2021-programmet og utlysningen av store, næringsrettede forskerprosjekter - 40 minutter
- Spørsmål og svar i plenum - 30 minutter
- Uformelle dialoger om prosjektmuligheter mellom de fremmøtte og med forskningsrådet etter behov og interesse.

Møtene er åpne for alle interesserte. Ingen påmelding.

### **Workshop Stochastic Demography in Fluctuating Environments: Theory and Empirical Patterns**

April 23-27, Helgeland, Norway. Aimed at young scientists in the initial stages of their scientific career

[More info](#)

### **International Summer School for Dynamics of Gene Regulation, Epigenetics and DNA Damage**

The IMB International Summer School (ISS) on the "Dynamics of Gene Regulation, Epigenetics and DNA Damage" is a 6-week programme for outstanding and enthusiastic undergraduate and Masters students who want to acquire excellent practical skills and hands-on training from leading scientists in molecular biology. If this describes you then please keep reading to find out more about the ISS and how to apply.

The 2012 ISS will take place from 16th July to 24th August and is coordinated by the Institute of Molecular Biology (IMB), a brand-new Centre of Excellence for Basic Research in the Life Sciences. IMB is located on the campus of the Johannes Gutenberg University Mainz and is funded by the Boehringer Ingelheim Foundation.

[More info](#)

### **Summer/spring courses Master and PhD in Svalbard**

Deadline for applying to summer/spring courses in Biology at the University Centre in Svalbard is now approaching. We have 4 courses for Master and PhD students offered. The deadline is 15<sup>th</sup> of February.

The courses available with application deadline 15.02.2012 is:

Flux of Matter and Energy from Sea to Land

Light Climate and Primary Productivity in the Arctic

Arctic Microbiology

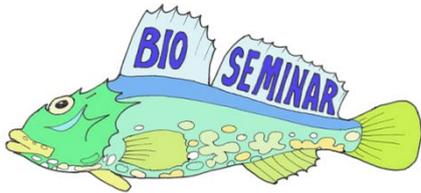
Ecosystems in Ice Covered Waters

[More info:](#)

### KOMMENDE MØTER OG SEMINAR

Mer info om kurs, møter, seminar og arrangement etc finner du [her](#).

BIO seminar Mikko Heino; Europeisk Marin Biologiske Konferansen i Arendal;



#### BIO seminar Mikko Heino

Torsdag 9. feb, kl 14.15-15 Seminarrom K1/K2, 1. etg A-blokk

#### **Fisheries-induced evolution: what we know, and what we would like to know?**

Summary : After research in fisheries-induced evolution intensified a little more than a decade ago, we have learned a lot and the field no longer is considered esoteric. In this presentation I describe why fisheries-induced evolution is an interesting and important research area from both academic and applied perspectives, and the key open questions that remain. Furthermore, I present two on-going projects, the guppy experiment at BIO and the analysis of skrei time series from Lofoten, that aim to fill some of these knowledge gaps.

**ALL BIO staff welcome!**



#### **Den 47. Europeiske Marinbiologiske konferanse**

Konferansen arrangeres i Arendal i begynnelsen av september. Vedlagt finner dere en [oppdatert poster \(A3-format\)](#) samt en presentasjon av "[keynote speakers](#)". Merk at datoen for "Abstract submission" er endret til 15.03.

#### **PhD MCB Introductory Course 2012, Glesvær, Norway, February 20-21, 2012**

The Molecular and Computational Biology Research School, MCB is a crossdisciplinary research school which has as it's main goal to "create an attractive and stimulating training environment for PhD students in molecular and computational biology, both to better serve the needs for relevant training in the field, and to stimulate crossdiscipline developments in the research of the parties".

MCB is a cooperative effort between 5 partner departments at the University of Bergen and Uni Research: Dept. of Biomedicine, Dept. of Biology, Department of informatics/CBU, Department of molecular biology and the Sars centre.

All PhD-students at any of the partner departments are welcome to join MCB.

The course is intended primarily for PhD-students who have started their PhD during the last year, or who are in the process of starting up at the moment. However, PhD-students in a later stage of their projects are also welcome to join, if they have not had the opportunity earlier. It is not a requirement that students are already admitted to the PhD-programme, so new PhD-fellows are welcome to join at an early stage.

Although any students are welcome to join, the scope of the school is molecular biology and computational biology. Thus, the course and membership to MCB is primarily relevant for students working within one or both of these areas.

For more information please see the MCB homepage: [www.uib.no/rs/mcb](http://www.uib.no/rs/mcb)

or contact us: [contact@mcb.uib.no](mailto:contact@mcb.uib.no)

tel: 55 58 45 03

Sign up: [contact@mcb.uib.no](mailto:contact@mcb.uib.no)

Deadline: February 10th

## NYE ARTIKLER

\*\*\*A full listing of BIO's ISI publications can be found on BIO's internal web pages. [Click here](#)

[Kirkendall](#); [Høisæter](#); [Olsen](#), [Schander](#), [Rapp](#); [Hejnol](#); [Tadiso](#); [Hordvik](#); [Rosland](#); [Roth](#); [Grimsbø](#); [Nortvedt](#)

Andersen HF, Jordal BH, Kambestad M, & **Kirkendall LR** (2012) Improbable but true: the invasive inbreeding ambrosia beetle *Xylosandrus morigerus* has generalist genotypes. *Ecology and Evolution* 2: 247-257.

**Abstract:** The wide distribution and dominance of invasive inbreeding species in many forest ecosystems seems paradoxical in face of their limited genetic variation. Successful establishment of invasive species in new areas is nevertheless facilitated by clonal reproduction: parthenogenesis, regular self-fertilization, and regular inbreeding. The success of clonal lineages in variable environments has been explained by two models, the frozen niche variation (FNV) model and the general-purpose genotype (GPG) model. We tested these models on a widely distributed forest pest that has been recently established in Costa Rica—the sibling-mating ambrosia beetle *Xylosandrus morigerus*. Two deeply diverged mitochondrial haplotypes coexist at multiple sites in Costa Rica. We find that these two haplotypes do not differ in their associations with ecological factors. Overall the two haplotypes showed complete overlap in their resource utilization; both genotypes have broad niches, supporting the GPG model. Thus, probable or not, our findings suggest that *X. morigerus* is a true ecological generalist. Clonal aspects of reproduction coupled with broad niches are doubtless important factors in the successful colonization of new habitats in distant regions.

**TØRE HØISÆTER** 2012. *Cimaria vargasi* n. gen, n. sp. (Gastropoda: Pyramidellidae: Odostomiinae) from the Pacific Coast of Costa Rica, Central America. *Zootaxa* 3178: 63-67 (2012)

**Olsen BR**, Dahlgren K, **Schander C**, Båmstedt U, **Rapp HT**, Troedsson C. Identification of specific prey items in the calanoid copepod *Limnocalanus macrurus* using Denaturing High-Performance Liquid Chromatography (DHPLC) assay. *Journal of Plankton Research* doi:10.1093/plankt/fbr110

**Abstract:** Denaturing high-performance liquid chromatography (DHPLC) is a relatively new method for separating amplicons in a mixture, and was recently developed for parasite detection in the blue crab *Callinectes sapidus*. That assay used a peptide nucleic acid (PNA) PCR hybridization blocking probe (PNA-PCR-DHPLC) to decrease the generic PCR bias of dominant templates (the host) in the mixture

prior to separation on the DHPLC column, thus enhancing the less abundant parasite DNA. The same assay and rationale can be used to investigate predator-prey interactions. However, in ecosystem studies with many predator-prey relationships, development of specific PNA-blocking probes for each predator would be too laborious. Here, we have developed a PCR-DHPLC assay excluding the dominant predator amplicons in a first DHPLC run, followed by re-amplification of the non-predator retention volumes and further separation and characterization in a second DHPLC run. This assay generated data on the specific trophic interactions between the calanoid copepod *Limnocalanus macrurus* and its prey from a seasonal sampling programme. The assay provides an efficient way for an unbiased screening of predator-prey relationships, and although developed for *L. macrurus* in this study, the approach has wide applicability for any predator-prey interaction

Scott Santagata, Carlee Resh, **Andreas Hejnol**, Mark Q Martindale and Yale J Passamaneck  
Development of the larval anterior neurogenic domains of *Terebratalia transversa* (Brachiopoda)

provides insights into the diversification of larval apical organs and the spiralian nervous system  
EvoDevo 2012, 3:3 doi:10.1186/2041-9139-3-3

**Background:** Larval features such as the apical organ, apical ciliary tuft, and ciliated bands often complicate the evaluation of hypotheses regarding the origin of the adult bilaterian nervous system. Understanding how neurogenic domains form within the bilaterian head and larval apical organ requires expression data from animals that exhibit aspects of both centralized and diffuse nervous systems at different life history stages. Here, we describe the expression of eight neural-related genes during the larval development of the brachiopod, *Terebratalia transversa*.

**Tadiso TM, Sharma A, Hordvik I** (2011) Analysis of polymeric immunoglobulin receptor- and CD300-like molecules from Atlantic salmon. *Molecular Immunology* 49:462-473

**Abstract:** The polymeric immunoglobulin receptor (pIgR) plays a pivotal role in mucosal immune protection by transporting secretory immunoglobulins to mucosal epithelia, and protecting them from proteolytic degradation. It has been reported that a homolog of the pIgR has a similar role in teleost fish. Considering the role pIgR has in mucosal defenses, this study was initiated to characterize a possible pIgR homolog in Atlantic salmon (*Salmo salar*) and its relatedness to pIgR of other vertebrates and similar molecules. Two pIgR-like cDNAs and genes of Atlantic salmon (Salsal pIgR and Salsal pIgRL) were cloned and analyzed. In addition, we gathered sequence information of CMRF35-like molecules (CLM) 1, 7, and 8 (designated as CD300 in humans) and made a comparative evaluation to that of the Salsal pIgR and Salsal pIgRL polypeptides. Salsal pIgR and Salsal pIgRL, like pIgR in other teleosts, are composed of two IG V domains, a connecting, a transmembrane, and a cytoplasmic region. The same holds true for Atlantic salmon CLM1 and CLM7, except that they possess putative immunoreceptor tyrosine-based inhibitory motifs (ITIM) in their cytoplasmic tails. The abundance of Salsal pIgR transcript is significantly higher than Salsal pIgRL and CLM in the skin, while Salsal pIgRL transcripts were abundant in the gills, depicting their possible tissue-specific role in mucosal immunity. To further highlight the roles of these molecules in cutaneous mucosal defence, we compared their transcriptional changes in salmon skin and spleen infected with the ectoparasite *Lepeophtheirus salmonis* which targets skin and mucus of salmonid fish (sampled 3, 14 and 28 days post infection (dpi)). Salsal pIgR and Salsal pIgRL transcripts significantly increased after 14 dpi in skin and spleen. CLM1 was up-regulated in skin and down-regulated in spleen, possibly indicating that CLM1 expressing cells had migrated to the target site. Homology modeling using human pIgR domain 1 (PDB 1xed) identified structurally equivalent residues on both Salsal pIgR and Salsal pIgRL, and the same domain disulphide bridge topology. Cysteines 42 and 50 (IMGT numbering) are 7 residues apart in all V domains of Salsal pIgR, Salsal pIgRL, and mammalian [D1].

**Rosland R, Bacher C, Strand O, Aure J, Strohmeier T** (2011) Modelling growth variability in longline mussel farms as a function of stocking density and farm design. *Journal of Sea Research* 66:318-330

**Abstract:** Mussels (*Mytilus edulis*) are commonly cultivated on artificial structures like rafts, poles or longlines to facilitate farming operations. Farm structures and dense mussel populations may result in water flow reduction and seston depletion and thus reduced individual mussel growth and spatial growth variability inside a farm. One of the challenges in mussel farming is thus to scale and configure farms in order to optimise total mussel production and individual mussel quality under different environmental regimes. Here we present a spatially resolved model for simulation of flow reduction, seston depletion and individual mussel growth inside a longline farm based on information about farm configuration (spacing between longlines, farm length and stocking density) and background environmental conditions (current speed, seston concentration and temperature). The model simulations are forced by environmental data from two fjords in south-western Norway and the farm configurations are defined within operational ranges.

The simulations demonstrate spatial growth patterns at longlines under environmental settings and farm configurations where flow reduction and seston depletion have significant impacts on individual mussel growth. Longline spacing has a strong impact on the spatial distribution of individual growth, and the spacing is characterised by a threshold value. Below the threshold growth reduction and spatial growth variability increase rapidly as a consequence of reduced water flow and seston supply rate, but increased filtration due to higher mussel densities also contributes to the growth reduction. The spacing threshold is moderated by other farm configuration factors and environmental conditions. Comparisons with seston depletion reported from other farm sites show that the model simulations are within observed ranges. A demonstration is provided on how the model can guide farm configuration

with the aim of optimising total farm biomass and individual mussel quality (shell length, flesh mass, spatial flesh mass variability) under different environmental settings. The model has a potential as a decision support tool in mussel farm management and will be incorporated into a GIS-based toolbox for spatial aquaculture planning and management.

Filgueira R, **Rosland R**, Grant J (2011) A comparison of scope for growth (SFG) and dynamic energy budget (DEB) models applied to the blue mussel (*Mytilus edulis*). *Journal of Sea Research* 66:403-410

**Abstract:** Growth of *Mytilus edulis* was simulated using individual based models following both Scope For Growth (SFG) and Dynamic Energy Budget (DEB) approaches. These models were parameterized using independent studies and calibrated for each dataset by adjusting the half-saturation coefficient of the food ingestion function term,  $X(K)$ , a common parameter in both approaches related to feeding behavior. Auto-calibration was carried out using an optimization tool, which provides an objective way of tuning the model. Both approaches yielded similar performance, suggesting that although the basis for constructing the models is different, both can successfully reproduce *M. edulis* growth. The good performance of both models in different environments achieved by adjusting a single parameter,  $X(K)$ , highlights the potential of these models for (1) producing prospective analysis of mussel growth and (2) investigating mussel feeding response in different ecosystems. Finally, we emphasize that the convergence of two different modeling approaches via calibration of  $X(K)$ , indicates the importance of the feeding behavior and local trophic conditions for bivalve growth performance. Consequently, further investigations should be conducted to explore the relationship of  $X(K)$  to environmental variables and/or to the sophistication of the functional response to food availability with the final objective of creating a general model that can be applied to different ecosystems without the need for calibration.

A. Foss, **E. Grimsbø**, E. Vikingstad, **R. Nortvedt**, E. Slinde and B. Roth Live chilling of Atlantic salmon: physiological response to handling and temperature decrease on welfare. *Fish Physiology and Biochemistry* DOI: 10.1007/s10695-011-9536-6

**Abstract:** Investigation of the physiological effects of live chilling in Atlantic salmon, *Salmo salar*, has been performed in two experiments. In the first, fish (mean weight 840 g) acclimatized to either 16, 8, or 4°C were directly transferred horizontally or vertically (9 combinations) to water temperatures of 16, 8, 4, or 0°C using a dip net. Blood samples were collected at 1 and 6 h (h) post-transfer. In the second experiment, fish (mean weight 916 g) acclimatized to 16°C were exposed to four temperature-drop regimes (no physical handling): 16–4°C (over 5 h), 16–4°C (over 1 h), 16–0°C (over 5 h), and 16–0°C (over 1 h). Blood samples were collected 1 h post-temperature drop. Physical transfers in the first trial, i.e., temperature drops, resulted in immediate (1 h) increases in blood lactate concentrations at all three temperatures, but levels were significantly reduced and close to pre-transfer levels after 6 h. Horizontal transfers, i.e., 16–16°C, 8–8°C, and 4–4°C, resulted in similar increases and were not significantly different from the groups exposed to temperature drops. The most severe vertical transfer (16–0) resulted in a swift loss of equilibrium and eventually death. In experiment 2, temperature drops from 16 to 4°C and from 16 to 0°C over a period of one or 5 h, without physically handling the fish, resulted in no significant increases in any of the measured parameters 1 h post-transfer, except in the 16–0 (1 h) group. The latter experienced a significant increase in blood sodium, glucose, lactate, and cortisol levels compared to all other groups. The results suggest that salmon are capable of tolerating relatively steep temperature drops without any significant negative effects on blood stress parameters and that physical stress from handling overrides the effect of thermal insults.

**Bjorn Roth, Endre Grimsbø**, Erik Slinde, Atle Foss, Lars Helge Stien, **Ragnar Nortvedt**. Crowding, pumping and stunning of Atlantic salmon, the subsequent effect on pH and rigor mortis.

*Aquaculture*. Volumes 326329, 25 January 2012, Pages 178180

**Abstract:** An optimum setting for electrical stunning of Atlantic salmon under commercial conditions was tested and compared against percussive stunning before and after pumping and at increasing durations of crowding (0.5 and >3 h). For electrical stunning the fish were exposed to 60 V, 100 Hz AC + DC for 6 s after pumping. The results show that there were no significant differences in muscle pH or rigor index between fish exposed to electricity or a percussive force. Pumping and crowding did have a significant effect. Pumping alone halved the time until maximum rigor from 24 to 12 h post mortem. Increasing crowding for 3 h caused a significant drop in muscle pH and the time until onset of maximum rigor tension was reduced to 6 h post mortem. We conclude that optimum electrical

stunning performs equally well as percussive stunning and that future challenges for improving the pre rigor times for Atlantic salmon lays in the pumping and crowding conditions, and not in the selected stunning technique.