Check for updates

*Journal of Fish Diseases*

WILEY

Journal of Fish Diseases

RESEARCH ARTICLE

# Efficacy of a Vaccine for Atlantic Salmon (*Salmo salar*) Using a *Tenacibaculum dicentrarchi* Strain Cultured Under Iron-Limited Conditions

Ruben Avendaño-Herrera[1,2,3] | Rute Irgang[1,2] | Henry Araya-León[1,2] | Pedro Ilardi[4] | Raúl Cortés[2]

[1]Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias de la Vida, Universidad Andrés Bello, Viña del Mar, Chile | [2]Centro Interdisciplinario Para la Investigación en Acuicultura (INCAR), Universidad Andrés Bello, Santiago, Chile | [3]Centro de Investigación Marina Quintay (CIMARQ), Universidad Andrés Bello, Santiago, Chile | [4]Cooperative Program for Aquaculture, Universidad de Chile, Universidad Católica del Norte, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile

**Correspondence:** Ruben Avendaño-Herrera (ravendano@unab.cl; reavendano@yahoo.com)

**Received:** 20 March 2025 | **Revised:** 18 June 2025 | **Accepted:** 20 June 2025

**Funding:** This study was supported by the Agencia Nacional de Investigación e Innovación through FONDECYT Grant No. 1190283, FONDAPINCAR Grant No. 1523A0007 and National Doctoral Scholarship Grant No. 2121395.

**Keywords:** bacterin-based vaccines | bath-challenged | relative percent survival | Tenacibaculosis

## ABSTRACT

Tenacibaculosis, caused by *T. dicentrarchi*, results in skin lesions, ulceration, yellow plaques on teeth, and haemorrhaging in the operculum, peduncle and pectoral fins. It is the second leading cause of mortality in farmed Atlantic salmon (*Salmo salar*) in Chile. Currently, no vaccine is available to prevent the disease. *In silico* and in vivo studies have shown that iron plays a key role in *T. dicentrarchi* infection. We hypothesised that culturing the TdCh05 vaccine strain under iron-limiting conditions (i.e., with non-assimilable iron chelator 2.2′-dipyridyl [DIP]) would enhance Atlantic salmon protection against tenacibaculosis. Fish were vaccinated intraperitoneally with prototypes (A) TdCh05, (B) TdCh05 + DIP or (C) A + B (a 1:1 mixture of inactivated cultures), and a control group was challenged by bath immersion with a heterologous *T. dicentrarchi* strain. Each prototype was emulsified with a commercial adjuvant. At 14 days post-challenge, Atlantic salmon injected with FMM broth had a cumulative mortality of 73.3%, followed by the TdCh05 + DIP (66.7%) and A + B (54.8%) prototypes. The lowest cumulative mortality value (50%) was observed for the prototype containing *T. dicentrarchi* TdCh05 grown only in FMM. Moreover, a high proportion of the vaccinated fish that survived the challenge, regardless of the vaccine prototype, carried *T. dicentrarchi* in various internal organs, particularly in the spleen. Future studies should focus on identifying the most suitable antigens to develop an effective vaccine for the prevention of *T. dicentrarchi*-induced tenacibaculosis.

## 1 | Introduction

Fish aquaculture is a highly important industry for Chile, second only to copper mining, and the country is recognised as one of the world's leading exporters of aquatic products, with a 4.5% share of global exports (FAO 2024). Three main species of salmonids are farmed: Atlantic salmon (*Salmo salar*), Coho salmon (*Oncorhynchus kisutch*) and rainbow trout (*O. mykiss*), with a harvested biomass of 1,107,109 tons of salmonids in 2023 (SERNAPESCA 2023). To achieve these production levels, fish farming is very intensive, which, as reported worldwide in all salmon-producing countries, creates a favourable environment for the emergence of new bacterial pathogens.

One of the main pathogens that poses a serious threat to Chilean salmonid aquaculture during the on-growing phase is

© 2025 John Wiley & Sons Ltd.

*Tenacibaculum dicentrarchi*, one of the seven members within the *Tenacibaculum* genus (referenced in Olsen et al. 2019, 2020), which cause diseases collectively known as tenacibaculosis. This disease has become the second leading infectious cause of death in Chilean farmed Atlantic salmon, with a mortality of 37.9% in 2023 (SERNAPESCA 2024). Tenacibaculosis in marine fish, as caused by *T. dicentrarchi*, is primarily characterised by skin erosions that may progress to ulceration. Affected areas can include the head, jaw and mouth, where yellow plaques appear on the teeth. Additionally, haemorrhaging may occur in the operculum, as well as in the peduncle and along the edges of the pectoral fins (Avendaño-Herrera et al. 2016; Klakegg et al. 2019).

Attempts at disease-outbreak control include the administration of medicated feed containing florfenicol, oxytetracycline and, in more severe cases, tiamulin (Irgang et al. 2021; Irgang and Avendaño-Herrera 2022), although efficacy is limited in most cases. While vaccination is the most effective approach to control tenacibaculosis caused by *Tenacibaculum maritimum* in turbot (*Scophthalmus maximus*) (Mabrok et al. 2023), no vaccine is currently available to prevent tenacibaculosis caused by *T. dicentrarchi* in Chilean aquaculture.

Numerous studies have been conducted to gain a better understanding of the diversity of *T. dicentrarchi* and its virulence mechanisms, particularly focusing on the role of iron during the infectious process. Initial *in silico* genome analysis of *T. dicentrarchi* has revealed a variety of genes encoding proteins associated with iron uptake mechanisms (Saldarriaga-Córdoba et al. 2021). Functional studies have shown that this fish pathogen employs multiple strategies for iron acquisition, including siderophore production and the utilisation of heme groups (Avendaño-Herrera et al. 2023). More recently, a proteomic analysis using the type strain CECT $7612^{T}$ and the Chilean strain TdCh05, grown under normal and iron-limited conditions to mimic the host environment, revealed a shared response to iron deprivation. Under iron limitation, this response included increased reductor metabolism, siderophore transport, heme compound synthesis, iron transporters and the expression of proteins associated with iron oxidation (Avendaño-Herrera et al. 2024).

Based on these previous studies, we hypothesised that culturing the *T. dicentrarchi* strain TdCh05 in a medium that mimics the in vivo environment, i.e., with iron limitation, may serve as an effective inactivated vaccine to protect farmed fish against tenacibaculosis. Considering that most existing vaccines for the prevention of bacterial diseases in fish are formulated as oil-based emulsions administered via intraperitoneal injection (Veenstra et al. 2017), the present study evaluated different injectable prototypes containing *T. dicentrarchi* antigens. In fact, there is evidence in the literature supporting the development of injectable vaccines to prevent external pathologies such as tenacibaculosis. For example, van Gelderen et al. (2009) used a vaccine based on inactivated *T. maritimum* with an adjuvant, achieving a 79.6% relative percent survival (RPS), demonstrating that protection can be achieved using inactivated, adjuvanted injectable vaccines.

Currently, the only registered vaccine for the prevention of *T. maritimum* is an injectable formulation known as Icthiovac-TM, commercially produced by the company HIPRA (Mabrok et al. 2023), for the prevention of tenacibaculosis in turbot (*Scophthalmus maximus*). However, vaccine efficacy may vary depending on the *Tenacibaculum* species or the isolate selected. For instance, Småge et al. (2018) tested a vaccine aimed at preventing tenacibaculosis caused by *T. finnmarkense*, observing high antibody titers at 8- and 12-weeks post-immunisation at high doses, although these antibody levels did not translate into protection when both homologous and heterologous challenge trials were conducted. Herein, we report the results obtained with a whole-cell vaccine prepared with *T. dicentrarchi* TdCh05 tested in three presentations and its effect on Atlantic salmon survival.

## 2 | Materials and Methods

### 2.1 | Bacterial Strain and Growth

The virulent *T. dicentrarchi* TdCh05 strain, isolated from a tail lesion of Atlantic salmon in 2014 (Avendaño-Herrera et al. 2016), was used in this study. This strain has been analysed genomically (Saldarriaga-Córdoba et al. 2021) and for virulence mechanisms related to iron uptake (Avendaño-Herrera et al. 2023, 2024) and outer membrane vesicles (Echeverría-Bugueño and Avendaño-Herrera 2024). The strain was routinely cultured on the *Flexibacter maritimus* medium (FMM) (Pazos et al. 1996) at 18°C for 48 h. Identification of the strain as *T. dicentrarchi* was confirmed through PCR-based analysis (Avendaño-Herrera et al. 2018), complemented by standard phenotyping for this bacterial species (Avendaño-Herrera et al. 2016; Piñeiro-Vidal et al. 2012). Stock cultures were preserved at −80°C in CRYOBANK tubes (Mast Group, UK) or FMM broth supplemented with 10% glycerol (vol/vol).

### 2.2 | Vaccine Preparation

For the antigen preparation, TdCh05 colonies were harvested from the FMM plates after 48 h, resuspended in 5 mL of saline solution (0.85% NaCl, w/v), and the cell density was adjusted to McFarland scale 0.5. The cell suspension was inoculated in 300 mL FMM broth prepared with half the minimum inhibitory concentration previously established by Avendaño-Herrera et al. (2023) for this strain; that is, 65 μM of the non-assimilable iron chelator 2.2′-dipyridyl Reagent Plus (DIP) (TCI Europe; condition TdCh05 + DIP). This chelator concentration was added to the medium 48 h before inoculation of the strain and was kept under agitation at 100 rpm to ensure homogenisation. Additionally, cultures of the TdCh05 strain were prepared in FMM broth without supplementation of the iron chelator (condition TdCh05) under the same conditions. Each culture was performed in triplicate. Once the TdCh05 + DIP and TdCh05 cultures were grown, 300 mL of each culture were inoculated in 1200 mL of fresh FMM broth and then incubated at 18°C for 48 h. Each culture was performed in triplicate.

Before inactivation, samples from each culture were collected to quantify the final bacterial concentration using simple staining and a microscopic count on a Leica DM2000 microscope (Leica Microsystems, Wetzlar, Germany) (cells/mL). Additionally, the



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Ruben Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

number of viable bacteria was quantified by counting colony-forming units per mL (CFU/mL) through plating 10-fold serial dilutions on FMM agar plates. All plates were incubated at 18°C for 7 days, and the CFU/mL were calculated. After 48 h of incubation, the cell cultures were inactivated with formalin (0.7% v/v) and incubated at 18°C for 72 h with gentle agitation. Cell inactivation was confirmed by streaking the cells onto FMM agar plates, which were incubated as previously described. Once bacterial growth was confirmed to be absent, each culture was adjusted to a final concentration of $1 \times 10^9$ cells/mL by centrifuging a fraction of each culture at 5000 rpm for 20 min at 4°C. Consequently, each vaccine contained a concentration of $1 \times 10^9$ cells/mL. The inactivated TdCh05 + DIP and TdCh05 cultures were then stored at 4°C until preparation of the experimental formulations.

A total of three experimental vaccine prototypes of *T. dicentrarchi* were prepared: (A) Prototype TdCh05, (B) Prototype TdCh05 + DIP and (C) Prototype A + B, which corresponded to a 1:1 mixture of each inactivated culture. Each prototype was emulsified with the adjuvant Montanide ISA 763 AVG 36017Z (Seppic, France) according to the manufacturer's instructions. The three prototypes were then stored at 4°C for 30 days before use.

## 2.3 | Challenge Assay

All experimental procedures and animal care were conducted in accordance with the ethical guidelines established by the Ethics Committee of the Universidad Andrés Bello, under approval accreditation number 005/2019. Unvaccinated Atlantic salmon ($n = 220$; 211.6 ± 45.26 g) with no prior history of disease were sourced from a commercial farm. Before transport, and in accordance with regulations by the National Fisheries and Aquaculture Service, the fish were certified free of any Chilean pathogens (*Piscirickettsia salmonis, Renibacterium salmoninarum*, *Flavobacterium psychrophilum*, *T. dicentrarchi*, *T. maritimum*, infectious salmon anaemia virus and infectious pancreatic necrosis virus) by a recognised diagnostic laboratory. Upon arrival at the experimental facility, Atlantic salmon were anaesthetised using Acuestrol (Isoeugenol 50%) at a dose of 10 mg/L and tagged with Visible Implant Elastomer tags (Northwest Marine Technology, USA) in different colours according to treatment, following the manufacturer's instructions. Each fish received a subcutaneous injection of the polymer mixture in the lower jaw and was randomly distributed among five tanks with aerated seawater (33 ppt) at 15°C ± 1°C for 14 days prior to vaccination. No mortalities were recorded during the acclimation period in seawater.

A total of five fish groups, each in duplicate with 22 fish per group, were used: three vaccinated groups, one FMM-broth control group and one non-injected control group. All fish were anaesthetised using Acuestrol (Isoeugenol 50%) at a dose of 10 mg/L, and each fish received an intraperitoneal injection (0.1 mL/100 g fish weight), labelled according to the applied treatment.

Fish were then kept at a density of 15 kg/m$^3$ in separate tanks according to each treatment, using a recirculating system with a biofilter. They were fed at 1.5% body weight, and the tank conditions were as follows: a seawater exchange flow 2 L/min; dissolved oxygen levels between 80 a 110%; a 12:12 light: dark photoperiod; and water temperature set to 15°C ± 1°C.

Forty days after vaccination (673°-days), the fish were transferred to a 600-l tank, and the vaccinated and FFM-injected groups l bath-challenged for five hours with a concentration of $1 \times 10^7$ cells/mL of a heterologous *T. dicentrarchi* isolate Td-025, as described in Avendaño-Herrera et al. (2016) and Irgang and (Avendaño-Herrera et al. 2020). This isolate was recovered from an outbreak that occurred at an Atlantic salmon farming centre in 2019. In addition, the non-injected control group was subjected to the same challenge conditions but without bacteria, in order to rule out the effect of manipulation on mortality. Subsequently, each fish group was distributed between two tanks at a density of 15 kg/m$^3$ for 14 days, maintaining the conditions described previously.

## 2.4 | Sampling Procedure

During the challenge period, up to three dead fish were sampled per day per treatment. All dead fish showed clinical signs of tenacibaculosis. From these fish, attempts were made to reisolate the bacteria from external (e.g., skin lesions and ulcers) and internal (e.g., kidney, liver and spleen) samples. These samples were streaked onto FMM agar plates and incubated aerobically at 18°C for one week. Additionally, the same tissue samples used for colony isolation were deposited in RNAlater (Thermo Fisher Scientific Inc.) for PCR analysis (Avendaño-Herrera et al. 2018). Once the challenge period concluded, surviving fish were sacrificed with an overdose of benzocaine (BZ-20, Veterquímica S.A.) and externally and internally sampled for PCR tissue analysis.

## 2.5 | *T. dicentrarchi* Detection by PCR

Colonies obtained from fish tissues were analysed by a PCR designed for *T. dicentrarchi*, as described by Avendaño-Herrera et al. (2018). Once colonies suspected to be *T. dicentrarchi* TdCh05 were detected, these were Gram-stained and prepared for PCR diagnosis. Briefly, DNA was extracted from the colonies with the InstaGene Matrix (Bio-Rad), and DNA from fish tissues was processed with the TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Additionally, the DNA of the isolated colonies was subjected to PCR-based diagnostic testing for other pathogens of the *Tenacibaculum* genus, including *T. maritimum* (Toyama et al. 1996), *T. piscium* (Saldarriaga-Córdoba et al. 2023) and *T. soleae* (García-González et al. 2011).

## 2.6 | Vaccine Efficacy and Statistical Analyses

Analyses were performed using the RStudio statistical package v1.2.1335, considering differences significant at a $p$-value $< 0.05$. The efficacy of each vaccine prototype was determined by calculating the RPS (Amend 1981). The following formulas were used:

$$RPS = \left(1 - \left(\frac{\%\,\text{vaccine mortality}}{\%\,\text{mortality nonvaccinated}}\right)\right) \times 100$$



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Ruben Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Additionally, RPS60 was calculated as this is an indicator that evaluates the mortality of a vaccinated group when the FFM-injected group reaches 60% mortality.

$$RPS60 = \left(1 - \left(\frac{\%\,\text{vaccine mortality on the day the control group reaches 60\,\% mortality.}}{60\,\%\,\text{mortality nonvaccinated}}\right)\right) \times 100$$

To determine the effectiveness of the different vaccine prototypes, Kaplan–Meier survival curves were constructed, with the mortality of each fish considered an event and survivors censored at the end. Furthermore, the Log-Rank test was used to identify differences between the curves (D'Arrigo et al. 2021; Schober and Vetter 2018).

## 3 | Results and Discussion

Tenacibaculosis caused by *T. dicentrarchi* results in economic and environmental impacts. We therefore aimed to evaluate the protective capacity of prototype vaccines by growing bacteria under iron-limiting conditions. Cultivation conditions significantly impact synthesis of the bacterial outer membrane and secreted proteins, which are critical antigens, thereby affecting the immunogenicity of bacterin-based vaccines (Cao et al. 2025). The TdCh05 strain of Atlantic salmon was selected for antigen production, as the presence of iron uptake mechanisms is well known, and its pathogenic potential has been demonstrated through immersion challenges in Atlantic salmon (65%) and rainbow trout (93%) (Avendaño-Herrera et al. 2016).

At 14 days post-challenge (dpc), Atlantic salmon injected with FMM broth had a cumulative mortality of 73.3%, followed by the TdCh05 + DIP (66.7%) and A + B (54.8%) prototypes. The lowest cumulative mortality value (50%) was observed for the prototype containing *T. dicentrarchi* TdCh05 grown only in FMM. Although the cumulative mortality in the FMM control group was higher than the expected 60%, it is important to highlight that mortality in vaccinated fish were consistently lower, regardless of the antigen used.

The bacterial concentration of the heterologous isolate of *T. dicentrarchi* used was adjusted to $1 \times 10^6$ cells/mL (lethal dose 60) for the bath challenge (equivalent to approximately $5 \times 10^5$ CFU/mL), aligning with proposals using the same pathogen in similar studies (Småge et al. 2018). However, the viability assessment conducted in the bath challenge showed a one-log increase in the concentration of viable bacteria (i.e., $1 \times 10^7$ CFU/mL) compared to the direct cell count. Some species of the *Tenacibaculum* genus, such as *T. maritimum*, form aggregates, which hinder the quantification of the inoculum in challenge studies (Mabrok et al. 2023). In fact, using the *T. dicentrarchi* strain CCCM21/136, which does not form aggregates, Kumanan et al. (2025) also reported a ±10% variation in the precise calculation of the *T. dicentrarchi* inoculum concentration when conducting experimental challenge trials on Chinook salmon (*Oncorhynchus tshawytscha*). It is important to highlight that, although the bath challenge model mimics natural infection, it is not the optimal approach for testing vaccine efficacy because fish are exposed to a high concentration of bacteria for a brief time, which does not accurately represent natural infection conditions (Karlsen et al. 2017; Småge et al. 2018). This approach is consistent with guidelines for pathogens responsible for causing ulcers and external damage in fish (Karlsen et al. 2017; Småge et al. 2018).

Regardless of the antigen used in each prototype, most mortalities occurred within the first 4 dpc; even in fish vaccinated with TdCh05 + DIP, all mortalities occurred by 3 dpc (Figure 1). This temporal mortality kinetics caused by *T. dicentrarchi* is consistent with what has been described in the literature (Avendaño-Herrera et al. 2016; Klakegg et al. 2019). It is well known that *T. dicentrarchi* can adhere and form biofilms within the first 24 h, followed by a decline over the next 96 h (Levipan et al. 2019). Additionally, this bacterium exhibits an attraction to the mucus of healthy Atlantic salmon (Echeverría-Bugueño et al. 2023). As expected, no mortalities occurred in the non-injected, non-challenged control fish, validating the handling of animals during the experiment.

The calculated RPS and RPS60 values were below those expected. Fish vaccinated with the prototype containing *T. dicentrarchi* TdCh05 antigens grown only in FMM were the highest (31.82% and 45.53%, respectively). The A + B prototype followed, with an RPS of 25.21% and an RPS60 of 16.62%. Finally, the prototype with the TdCh05 strain grown with the iron chelator presented values of only 9.08% and 10.75%, respectively. Subsequent analyses using the Kaplan–Meier survival function revealed that only the TdCh05 prototype differed significantly from the FMM control group (Figure 2), exhibiting a statistically higher survival probability. The results of the non-parametric Log-Rank further supported these findings when comparing the different groups of Atlantic salmon vaccinated with various prototypes (Figure 2).

These vaccination results are unexpected, given that bacteria in the host can acquire iron during infection via siderophores, which bind and transport iron (Eichenbaum et al. 1996; Miethke and Marahiel 2007). Therefore, we anticipated that the presence of the chelating agent 2,2′-dipyridyl in the culture medium would significantly influence the production of many iron-related proteins in *T. dicentrarchi* that are not expressed under normal growth conditions (Avendaño-Herrera et al. 2023, 2024). These proteins could be immunogenic and enhance the protective response of fish vaccinated with the TdCh05 + DIP prototype. However, completely opposite results were obtained, with RPS values as low as approximately 10%. Recently, Cao et al. (2025) reported that lumpfish (*Cyclopterus lumpus*) injected with bacterin derived from iron-supplemented cultures at 15°C showed an RPS of 14.29% when challenged with *Vibrio anguillarum*. However, bacterin-based vaccines derived from *V. anguillarum* cultured under iron-limited conditions with 2% NaCl at 28°C provided significantly higher protection compared to those prepared under iron-rich conditions. To date, it remains unclear how these culture conditions affect the immunogenicity of *T. dicentrarchi*.



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Ruben Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

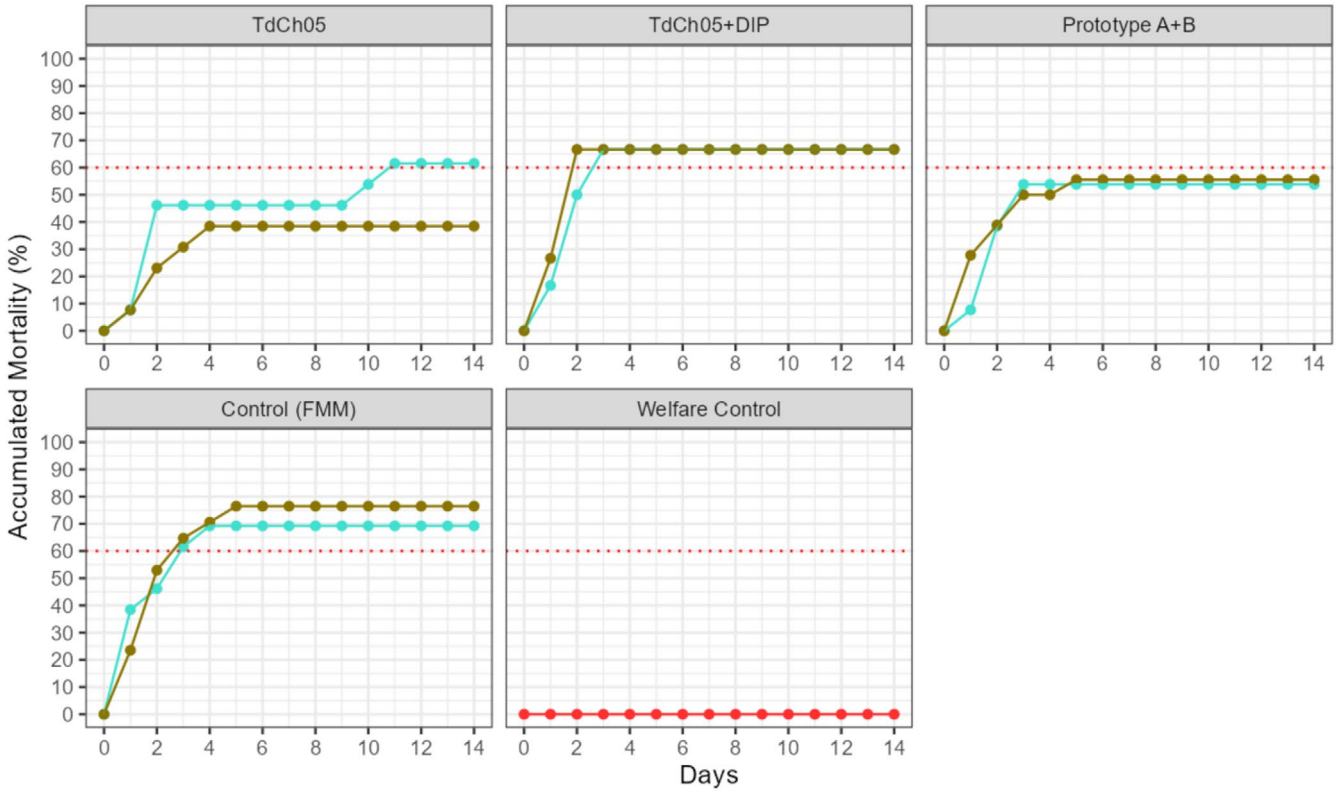


**FIGURE 1** | Accumulated mortalities of Atlantic salmon challenged with a bath containing a concentration of $1 \times 10^7$ cells/mL of a heterologous *T. dicentrarchi* isolate, 40 days post-vaccination. Three fish groups in duplicate (22 fish per replicate) were vaccinated with (A) Prototype TdCh05, (B) Prototype TdCh05 + DIP or (C) Prototype A + B, which corresponded to a 1:1 mixture of each inactivated culture. Additionally, one FMM-broth control group, and one non-injected control group (welfare control) were included. Each prototype was emulsified with the adjuvant Montanide ISA 763 AVG 36017Z (Seppic, France).

On the other hand, vaccination studies with phylogenetically related pathogens such as *T. maritimum* and *T. finnmarkense* indicate that these bacteria induce little to no inflammatory response (Evelyn et al. 1992; Småge et al. 2018). Even when an antibody response is present in Atlantic salmon, Frisch et al. (2018), when evaluating an inactivated whole-cell vaccine with an oil-based adjuvant against *T. maritimum*, did not observe significant protection. Småge et al. (2018) suggest that the rapid progression of infection with *Tenacibaculum* spp. may contribute to the lack of protection, as the immune system of fish might not have sufficient time to respond. Ongoing field and in vivo studies are being conducted to analyse the immune response of fish and determine if a specific immune response is induced following challenges with *T. dicentrarchi*.

The infected fish in this experiment exhibited clinical signs similar to those observed during natural outbreaks of tenacibaculosis in the field. To confirm that the mortalities of the vaccinated Atlantic salmon were specifically caused by the employed heterologous isolate of *T. dicentrarchi*, bacteriological isolation was performed from various organs and then confirmed using a PCR (Table 1). Sixty-one isolates resembling *T. dicentrarchi* were confirmed positive as *T. dicentrarchi* TdCh05, amplifying the 284 bp product.

When analysed by challenge prototype, samples from 9 of 13 fish in the FMM control group presented at least one *T. dicentrarchi* colony (Table 1). For two of these fish (No. 1 and 12), *T. dicentrarchi* was detected in external lesions and internal organs (i.e., spleen, liver and kidney). For the TdCh05 prototype, at least one sample from 6 of 16 fish had *T. dicentrarchi*-positive colonies. Furthermore, in two fish (No. 1 and 2) collected early in the challenge, the bacterium was isolated from external lesions and internal organs (i.e., spleen, liver and kidney). In the case of fish vaccinated with TdCh05 + DIP, at least one sample from 6 of 11 fish was positive for *T. dicentrarchi*. In fact, in three fish (No. 1, 2 and 3) that died within the first 2 dpc, *T. dicentrarchi* was detected in spleen, liver, kidney and skin-lesion samples. When the microbiological analysis was performed on the mortalities of fish vaccinated with the antigen mixture (A + B), at least one sample from 7 out of 13 Atlantic salmon were PCR-positive for *T. dicentrarchi*. Additionally, in three of the fish (No. 1, 2 and 3), bacteria were detected in external lesions, as well as the spleen, liver and kidney.

It is important to note that most positive PCR reactions from colonies were obtained from TdCh05 isolated from liver samples (32.8%). No DNA from the 49 bacterial colonies amplified for *T. maritimum* (Toyama et al. 1996), *T. piscium* (Saldarriaga-Córdoba et al. 2023) or *T. soleae* (García-González et al. 2011).

During the euthanasia of live fish at the conclusion of the experiment, tissue samples were collected from seven fish in the FMM control group. PCR results were positive in five out of the seven fish, with at least one of the extracted DNA samples from the analysed organs testing positive. Only one fish tested positive



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Ruben Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

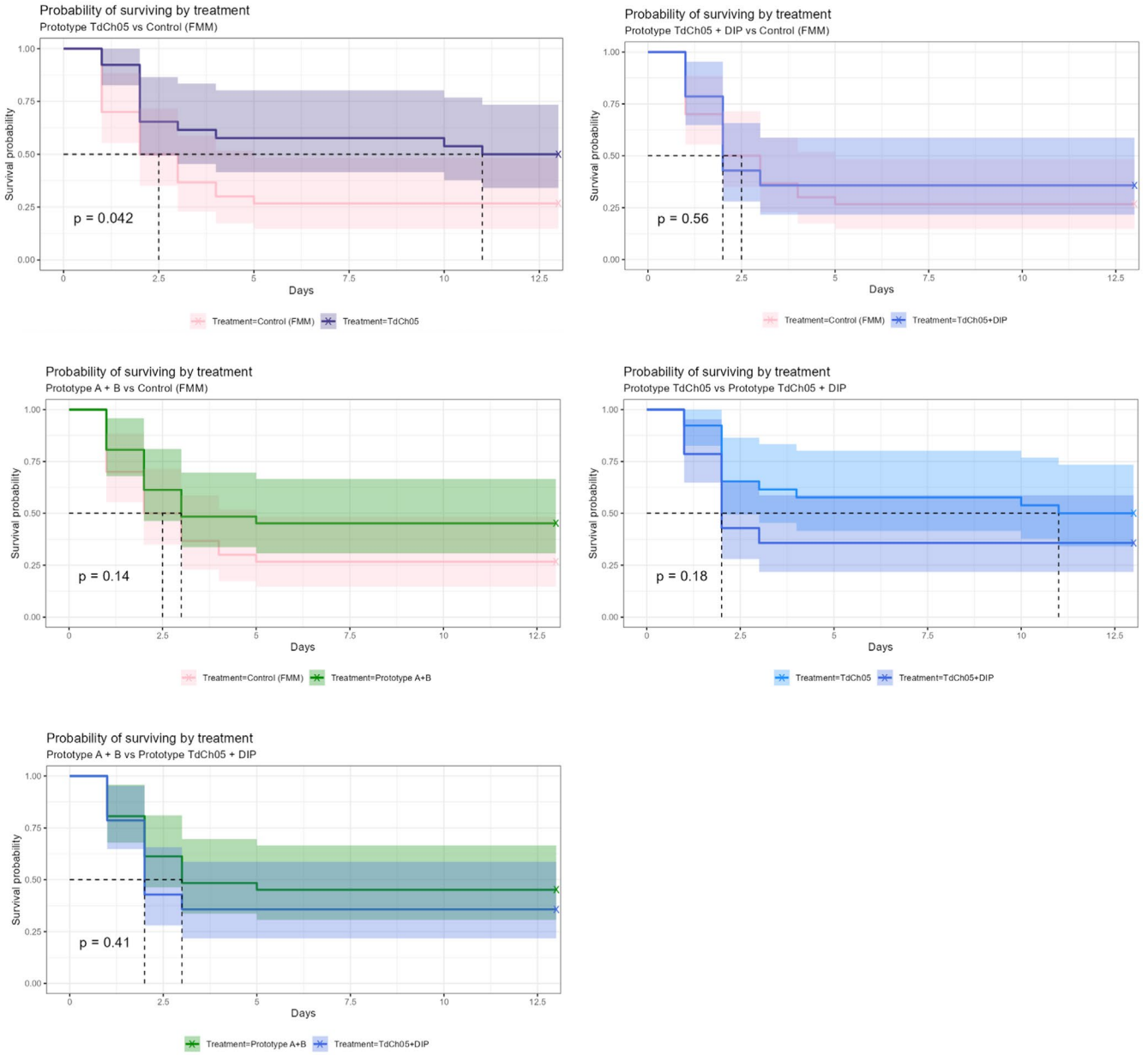


**FIGURE 2** | Survival probability of Atlantic salmon vaccinated with different prototypes, as well as the unvaccinated control groups, subjected to an immersion challenge with a heterologous *T. dicentrarchi* isolate. In each graph, comparisons are made between the different treatments and/or controls. The dashed line indicates the day at which 50% survival was reached for each group, while the *p*-value represents the probability. The darkest area of each Kaplan–Meier curve represents the confidence interval calculated using the 'survminer' package used to generate the curves. The horizontal dashed line indicates 50% survival, and the vertical dashed line marks the day on which each curve reached a 50% probability of survival.

in the skin, spleen and liver. Regarding fish vaccinated with the TdCh05 prototype, at least one tissue sample from nine out of ten surviving fish analysed tested positive for *T. dicentrarchi* by PCR. For the fish vaccinated with the TdCh05 + DIP prototype, tissue samples were obtained from seven fish euthanised at the end of the challenge. The bacterium was detected in at least one sample from six of these fish, with only one fish testing PCR-positive for all the extracted tissue samples. Finally, in the A + B mix group, tissue samples were collected from seven fish euthanised at the end of the challenge, all of which tested positive by PCR in at least one of the analysed samples. It is interesting to note that regardless of the treatment the fish underwent, the tissue with the highest PCR positivity was the spleen (22 out of the 31 fish analysed) (Figure 3).

As observed, *T. dicentrarchi* was predominantly recovered in cultures from microbiological samples of internal organs, contrary to the negative results reported in other studies (Avendaño-Herrera et al. 2020; Kumanan et al. 2025), where isolation was more successful from the skin and gills. Moreover, Irgang and Avendaño-Herrera (2021) noted that isolating *T. dicentrarchi* on FMM plates is not always successful, even when fish show external injuries. Furthermore, the obtained PCR results in the present study from various tissues of the surviving fish were consistent with the microbiological diagnoses (Figure 3). It is important to highlight that the key difference from previous studies, which may explain the successful isolation of the bacterium from internal organs, was that the Atlantic salmon were analysed microbiologically immediately. This was possible



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Ruben Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

**TABLE 1** | Isolation of *T. dicentrarchi* bacterial colonies recovered from mortalities in fish vaccinated with the three vaccine prototypes and the control group (FMM medium), which were confirmed as positive by species-specific PCR. '+', positive diagnosis for *T. dicentrarchi*. The number of fish (No.) varied for each treatment and control group. Empty cells indicate no isolation of *T. dicentrarchi* colonies.

| **Prototype** | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **FMM** | | | | | **TdCh05** | | | | | **TdCh05 + DIP** | | | | | **A + B** | | | | |
| **Fish No.** | **spleen** | **liver** | **kidney** | **skin** | **Fish No.** | **spleen** | **liver** | **kidney** | **skin** | **Fish No.** | **spleen** | **liver** | **kidney** | **skin** | **Fish No.** | **spleen** | **liver** | **kidney** | **skin** |
| 1 | + | + | + | + | 1 | + | + | + | + | 1 | + | + | + | + | 1 | + | + | + | + |
| 2 | | + | | + | 2 | + | + | + | + | 2 | + | + | + | + | 2 | + | + | + | + |
| 3 | | | | | 3 | | | | | 3 | + | + | + | + | 3 | + | + | + | + |
| 4 | | + | | | 4 | | | | | 4 | | | | | 4 | | | | |
| 5 | | | + | | 5 | | + | | | 5 | + | | + | | 5 | + | | | |
| 6 | | + | | | 6 | | | | | 6 | | + | | | 6 | | | | |
| 7 | | | | + | 7 | | | | | 7 | | + | | | 7 | | + | | |
| 8 | | | | | 8 | | + | | | 8 | | | | | 8 | | | | |
| 9 | | + | | + | 9 | | | | | 9 | | | | | 9 | | | | |
| 10 | + | | + | | 10 | | | | | 10 | | | | | 10 | | + | | |
| 11 | | | | | 11 | | | | | 11 | | | | | 11 | | | | |
| 12 | + | + | + | + | 12 | | | | | | | | | | 12 | | | | |
| 13 | | | | | 13 | | | | | | | | | | 13 | + | | | |
| | | | | | 14 | | | | + | | | | | | | | | | |
| | | | | | 15 | | | | + | | | | | | | | | | |
| | | | | | 16 | | | | | | | | | | | | | | |
| **No. of colonies per sampled organ in fish** | | | | | | | | | | | | | | | | | | | |
| Total | 3 | 6 | 4 | 5 | | 2 | 4 | 2 | 4 | | 4 | 5 | 4 | 3 | | 5 | 5 | 3 | 3 |



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Rubén Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

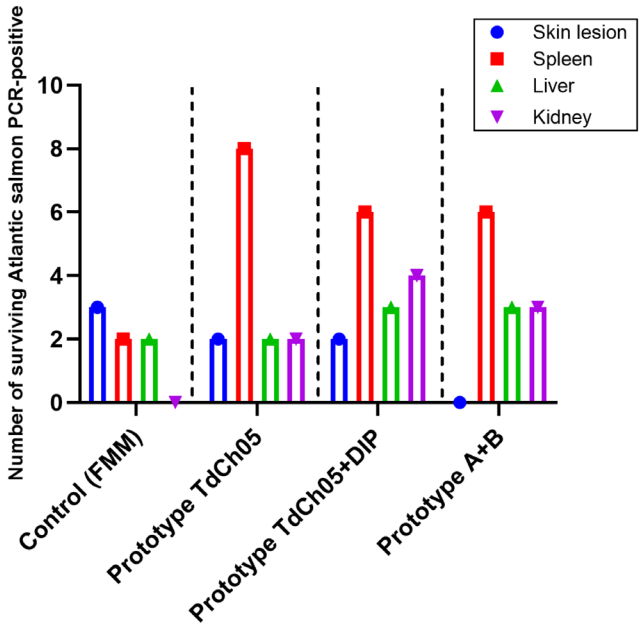


**FIGURE 3** | PCR diagnosis of positivity in vaccinated and control fish that survived the *T. dicentrarchi* challenge according to the analysed tissue.

because the facilities had the necessary infrastructure for sampling, and the temporal mortality kinetics were well understood. Another factor to consider in the successful diagnosis of internal organs is that the challenge was carried out using a higher concentration than those used in previous studies. In this sense, the possibility cannot be excluded that non-detection in internal organs in previous studies was because tenacibaculosis is primarily an external infection.

## 4 | Conclusion

The present study provides valuable insights into the development of vaccines against *T. dicentrarchi* in Atlantic salmon. Our results clearly showed that the *T. dicentrarchi* TdCh05 vaccine prototype, grown under iron-limiting conditions, is not effective in protecting Atlantic salmon against tenacibaculosis. In contrast, the whole-cell inactivated vaccine prototype TdCh05, grown in FMM, could provide adequate protection if the bacterial challenge concentration is lower than that used in this study or similar to the natural conditions to which fish are exposed in a farming facility. Additionally, it was demonstrated that a high proportion of vaccinated fish that survived the challenge, regardless of the vaccine prototype, carried *T. dicentrarchi* in various internal organs, particularly in the spleen. Future studies should focus on generating information about the immune response of vaccinated fish to identify an effective vaccine for the prevention of *T. dicentrarchi*-induced tenacibaculosis.

### Author Contributions

**Ruben Avendaño-Herrera:** conceptualization, visualization, writing – original draft, supervision, project administration, writing – review and editing, funding acquisition, resources, validation, supervision, project administration. **Rute Irgang:** data curation, formal analysis, investigation, methodology, software, writing – original draft. **Henry Araya-León:** formal analysis, investigation, methodology. **Pedro Ilardi:** writing – original draft, investigation, methodology. **Raúl Cortés:** writing – originaldraft, data curation, formal analysis, writing – review and editing.

### Acknowledgements

This study was supported by the Agencia Nacional de Investigación e Innovacion through FONDECYT Grant No. 1190283, FONDAPINCAR Grant No. 1523A0007 and National Doctoral Scholarship Grant No. 2121395. PI acknowledges the Deputy Directorate of Human Capital at ANID for the National Doctoral Scholarship No. 2121395.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

Data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

### References

Amend, D. F. 1981. "Potency Testing of Fish Vaccines." *Developments in Biological Standardization* 49: 447–454.

Avendaño-Herrera, R., C. Collarte, M. Saldarriaga-Córdoba, and R. Irgang. 2020. "New Salmonid Hosts for Tenacibaculum Species: Expansion of Tenacibaculosis in Chilean Aquaculture." *Journal of Fish Diseases* 43, no. 9: 1077–1085. https://doi.org/10.1111/jfd.13213.

Avendaño-Herrera, R., M. Echeverría-Bugueño, M. Hernández, P. Saldivia, and R. Irgang. 2024. "Proteomic Characterization of *Tenacibaculum dicentrarchi* Under Iron Limitation Reveals an Upregulation of Proteins Related to Iron Oxidation and Reduction Metabolism, Iron Uptake Systems and Gliding Motility." *Journal of Fish Diseases* 47, no. 9: e13984. https://doi.org/10.1111/jfd.13984.

Avendaño-Herrera, R., R. Irgang, C. Sandoval, et al. 2016. "Isolation, Characterization and Virulence Potential of *Tenacibaculum dicentrarchi* in Salmonid Cultures in Chile." *Transboundary and Emerging Diseases* 63: 121–126. https://doi.org/10.1111/tbed.12464.

Avendaño-Herrera, R., R. Irgang, and D. Tapia-Cammas. 2018. "PCR Procedure for Detecting the Fish Pathogen *Tenacibaculum dicentrarchi*." *Journal of Fish Diseases* 41: 715–719. https://doi.org/10.1111/jfd.12767.

Avendaño-Herrera, R., M. Saldarriaga-Córdoba, M. Echeverría-Bugueño, and R. Irgang. 2023. "In Vitro Phenotypic Evidence for the Utilization of Iron From Different Sources and Siderophore Production in the Fish Pathogen *Tenacibaculum dicentrarchi*." *Journal of Fish Diseases* 46, no. 9: 1001–1012. https://doi.org/10.1111/jfd.13820.

Cao, T., H. Gnanagobal, M. Dang, et al. 2025. "Influence of *Vibrio anguillarum* Culture Conditions on the Efficacy of Bacterin-Based Vaccines in Lumpfish (*Cyclopterus lumpus*)." *Fish & Shellfish Immunology* 158: 110133. https://doi.org/10.1016/j.fsi.2025.110133.

D'Arrigo, G., D. Leonardis, S. Abd ElHafeez, M. Fusaro, G. Tripepi, and S. Roumeliotis. 2021. "Methods to Analyse Time-to-Event Data: The Kaplan-Meier Survival Curve." *Oxidative Medicine and Cellular Longevity* 2021: 2290120. https://doi.org/10.1155/2021/2290120.

Echeverría-Bugueño, M., and R. Avendaño-Herrera. 2024. "*Tenacibaculum dicentrarchi* Produce Outer Membrane Vesicles (OMV) That Are Associated With the Cytotoxic Effect in Rainbow Trout Head Kidney Macrophages." *Journal of Fish Diseases* 47, no. 2: e13888. https://doi.org/10.1111/jfd.13888.

Echeverría-Bugueño, M., R. Irgang, J. Mancilla-Schulz, and R. Avendaño-Herrera. 2023. "Healthy and Infected Atlantic Salmon



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Ruben Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

(*Salmo salar*) Skin-Mucus Response to *Tenacibaculum dicentrarchi* Under In Vitro Conditions." *Fish & Shellfish Immunology* 136: 108747. https://doi.org/10.1016/j.fsi.2023.108747.

Eichenbaum, Z., E. Muller, S. A. Morse, and J. R. Scott. 1996. "Acquisition of Iron From Host Proteins by the Group A Streptococcus." *Infection and Immunity* 64, no. 12: 5428–5429. https://doi.org/10.1128/iai.64.12.5428-5429.1996.

Evelyn, T. P. T., M. L. Kent, and T. T. Poppe. 1992. "Bacterial Diseases." In *Diseases of Seawater Netpen-Reared Salmonid Fishes in the Pacific Northwest*, edited by M. L. Kent and T. T. Poppe, 17–35. *Canadian Special Publication of Fisheries and Aquatic Sciences*.

FAO. 2024. "El Estado Mundial de la Pesca y la Acuicultura 2024. La Transformación Azul en Acción."

Frisch, K., S. B. Småge, C. Vallestad, and H. Duesund. 2018. "Experimental Induction of Mouthrot in Atlantic Salmon Smolts Using *Tenacibaculum maritimum* From Western Canada." *Journal of Fish Diseases* 41: 1247–1258. https://doi.org/10.1111/jfd.12818.

García-González, P., N. García-Lamas, C. Fuentes Edfuf, and Y. Santos. 2011. "Development of a PCR Method for the Specific Identification of the Marine Fish Pathogen *Tenacibaculum soleae*." *Aquaculture* 319, no. 1–2: 1–4. https://doi.org/10.1016/j.aquaculture.2011.06.013.

Irgang, R., and R. Avendaño-Herrera. 2021. "Experimental Tenacibaculosis Infection in Adult Conger Eel (*Genypterus chilensis*, Guichenot 1948) by Immersion Challenge With *Tenacibaculum dicentrarchi*." *Journal of Fish Diseases* 44, no. 2: 211–216. https://doi.org/10.1111/jfd.13282.

Irgang, R., and R. Avendaño-Herrera. 2022. "Evaluation of the In Vitro Susceptibility of *Tenacibaculum dicentrarchi* to Tiamulin Using Minimum Inhibitory Concentration Tests." *Journal of Fish Diseases* 45, no. 6: 795–799. https://doi.org/10.1111/jfd.13604.

Irgang, R., M. Mancilla, and R. Avendaño-Herrera. 2021. "Florfenicol and Oxytetracycline Susceptibility Patterns in Chilean Isolates of *Tenacibaculum dicentrarchi*: An Emerging Pathogen for Farmed Salmonids." *Journal of Fish Diseases* 44, no. 8: 1043–1046. https://doi.org/10.1111/jfd.13380.

Karlsen, C., R. Thorarinsson, C. Wallace, K. Salonius, and P. J. Midtlyng. 2017. "Atlantic Salmon Winter-Ulcer Disease: Combining Mortality and Skin Ulcer Development as Clinical Efficacy Criteria Against *Moritella viscosa* Infection." *Aquaculture* 473: 538–544. https://doi.org/10.1016/j.aquaculture.2017.01.035.

Klakegg, Ø., T. Abayneh, A. K. Fauske, M. Fülberth, and H. Sørum. 2019. "An Outbreak of Acute Disease and Mortality in Atlantic Salmon (*Salmo salar*) Post-Smolts in Norway Caused by *Tenacibaculum dicentrarchi*." *Journal of Fish Diseases* 42, no. 6: 789–807. https://doi.org/10.1111/jfd.12982.

Kumanan, K., J. Carson, R. B. J. Hunter, et al. 2025. "Experimental Challenge of Chinook Salmon (*Oncorhynchus tshawytscha*) With Tenacibaculum Maritimum and *Tenacibaculum dicentrarchi* Fulfils Koch's Postulates." *Journal of Fish Diseases* 48, no. 6: e14088. https://doi.org/10.1111/jfd.14088.

Levipan, H. L., R. Irgang, D. Tapia-Cammas, and R. Avendaño-Herrera. 2019. "A High-Throughput Analysis of Biofilm Formation by the Fish Pathogen *Tenacibaculum dicentrarchi*." *Journal of Fish Diseases* 42, no. 4: 617–621. https://doi.org/10.1111/jfd.12949.

Mabrok, M., A. M. Algammal, E. Sivaramasamy, et al. 2023. "Tenacibaculosis Caused by *Tenacibaculum maritimum*: Updated Knowledge of This Marine Bacterial Fish Pathogen." *Frontiers in Cellular and Infection Microbiology* 12: 1068000. https://doi.org/10.3389/fcimb.2022.1068000.

Miethke, M., and M. A. Marahiel. 2007. "Siderophore-Based Iron Acquisition and Pathogen Control." *Microbiology and Molecular Biology Reviews* 71: 413–451. https://doi.org/10.1128/MMBR.00012-07.

Olsen, A. B., J. Powell, A. Siah, D. J. Colquhoun, and R. Avendaño-Herrera. 2019. "Tenacibaculosis." In *Technical Report: An Overview of Emerging Diseases in Salmonid Farming Industry*, edited by E. Brun, J. F. Rodríguez, and A. Macdonald, 43–49. ELANCO.

Olsen, A. B., B. Spilsberg, H. K. Nilsen, et al. 2020. "*Tenacibaculum piscium* sp. Nov., Isolated From Skin Ulcers of Sea-Farmed Fish, and Description of *Tenacibaculum finnmarkense* sp. Nov. With Subdivision Into Genomovars *Finnmarkense* and *Ulcerans*." *International Journal of Systematic and Evolutionary Microbiology* 70, no. 12: 6079–6090. https://doi.org/10.1099/ijsem.0.004501.

Pazos, F., Y. Santos, A. R. Macias, S. Nuñez, and A. E. Toranzo. 1996. "Evaluation of Media for the Successful Culture of *Flexibacter maritimus*." *Journal of Fish Diseases* 19: 193–197. https://doi.org/10.1111/j.1365-2761.1996.tb00701.x.

Piñeiro-Vidal, M., D. Gijón, C. Zarza, and Y. Santos. 2012. "*Tenacibaculum dicentrarchi* Sp. Nov., a Marine Bacterium of the Family Flavobacteriaceae Isolated From European Sea Bass." *International Journal of Systematic and Evolutionary Microbiology* 62, no. 2: 425–429. https://doi.org/10.1099/ijs.0.025122-0.

Saldarriaga-Córdoba, M., H. Araya-León, R. Irgang, and R. Avendaño-Herrera. 2023. "Development of a PCR Assay for the Specific Identification of the Fish Pathogen Tenacibaculum Piscium." *Journal of Fish Diseases* 46, no. 5: 517–526. https://doi.org/10.1111/jfd.13764.

Saldarriaga-Córdoba, M., R. Irgang, and R. Avendaño-Herrera. 2021. "Comparison Between Genome Sequences of Chilean *Tenacibaculum dicentrarchi* Isolated From Red Conger Eel (*Genypterus chilensis*) and Atlantic Salmon (*Salmo salar*) Focusing on Bacterial Virulence Determinants." *Journal of Fish Diseases* 44, no. 11: 1843–1860. https://doi.org/10.1111/jfd.13503.

Schober, P., and T. R. Vetter. 2018. "Survival Analysis and Interpretation of Time-To-Event Data: The Tortoise and the Hare." *Anesthesia and Analgesia* 127, no. 3: 792–798. https://doi.org/10.1213/ANE.0000000000003653.

SERNAPESCA. 2023. "Informe Sobre uso de Antimicrobianos y Antiparasitarios en la Salmonicultura Nacional 2023." https://www.sernapesca.cl/app/uploads/2024/06/Informe-sobre-el-uso-deantimicrobianos-y-antiparasitarios-en-la-salmonicultura-nacional-Ano-2023_v20240606.pdf.

SERNAPESCA. 2024. "Informe con Antecedentes Sanitarios de Agua Dulce y mar 2024." https://www.sernapesca.cl/app/uploads/2024/09/Informe-Sanitario-ANO-2023.pdf.

Småge, S. B., K. Frisch, V. Volda, et al. 2018. "Induction of Tenacibaculosis in Atlantic Salmon Smolts Using Tenacibaculum Finnmarkense and the Evaluation of a Whole Cell Inactivated Vaccine." *Aquaculture* 495: 858–864. https://doi.org/10.1016/j.aquaculture.2018.06.063.

Toyama, T., K. Kita-Tsukamoto, and H. Wakabayashi. 1996. "Identification of *Flexibacter maritimus*, Flavobacterium Branchiophilum and *Cytophaga columnaris* by PCR Targeted 16S Ribosomal DNA." *Fish Pathology* 31: 25–31. https://doi.org/10.3147/jsfp.31.25.

van Gelderen, R., J. Carson, B. Nowak, et al. 2009. "Experimental Vaccination of Atlantic Salmon (*Salmo salar* l.) Against Marine Flexibacteriosis." *Aquaculture* 288: 7–13. https://doi.org/10.1016/j.aquaculture.2008.11.012.

Veenstra, K. A., T. Wang, A. Alnabulsi, et al. 2017. "Analysis of Adipose Tissue Immune Gene Expression After Vaccination of Rainbow Trout With Adjuvanted Bacterins Reveals an Association With Side Effects." *Molecular Immunology* 88: 89–98. https://doi.org/10.1016/j.molimm.2017.05.026.



13652761, 2026, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/jfd.70013 by Ruben Avendaño-Herrera - Universidad Andres Bello , Wiley Online Library on [26/01/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License