

FISH HEALTH

Greek farmers spot diseases early with diagnostic kits

reports Dr Panos Varvarigos, Director of Vet Care Ltd

BACTERIAL disease outbreaks, especially in the form of acute larval mortalities in the hatcheries or sudden mortalities without particular disease symptoms of on-growing sea bream, sea bass and trout, are costly problems on Greek fish farms, particularly following stock handling or the influence of natural stressors.

In such cases, urgent action needs to be taken to prevent deaths escalating among the other, apparently healthy, stock.

Measures include mobilising professional assistance to identify the causative agent, usually by limited sampling of suspect fish tissues for bacteriological examination. But the process of culture incubation, purification and further

biochemical testing to identify the implicated bacterium is not only costly, but, more important, it is time consuming. This is further complicated by the fact that many of the disease causing bacteria are difficult to isolate and grow slowly, requiring special growth media.

Prime examples are the causative agent of bacterial kidney disease (BKD), *Renibacterium salmoninarum*, in freshwater water fish, which requires more than a week at 17-20°C to grow on Kidney Disease Medium-2; and

Pasteurella piscicida, the causative agent of Pasteurellosis in saltwater fish, which is particularly serious in the larval stage of sea bream and during the early on-growing stage of sea bass.

The Aquarapid range of kits provide for the testing of 40 samples against a range of major pathogens.

Pasteurella requires at least three days incubation at 25°C on marine agar medium prior to producing visible colonies. Even then the colonies may not be pure since other present bacteria (such as the *Vibrios*) interfere requiring further efforts to isolate the bacterial strain and test its sensitivity against the available antibiotics.

Evidently, the major hurdles are, firstly, the lack of timely information on identification of the cause of the disease and, secondly, the

limited number of samples that may be examined in each case.

Therefore, the immediate therapeutic action to prevent the spread of the disease lacks the much needed confirmed laboratory information and so antibiotics may be administered according to treatment schedules which at best are "educated guesses" based on the past history of the site. Also, the disease profile or health status of the fish population, or at least parts of it, cannot be elabo-

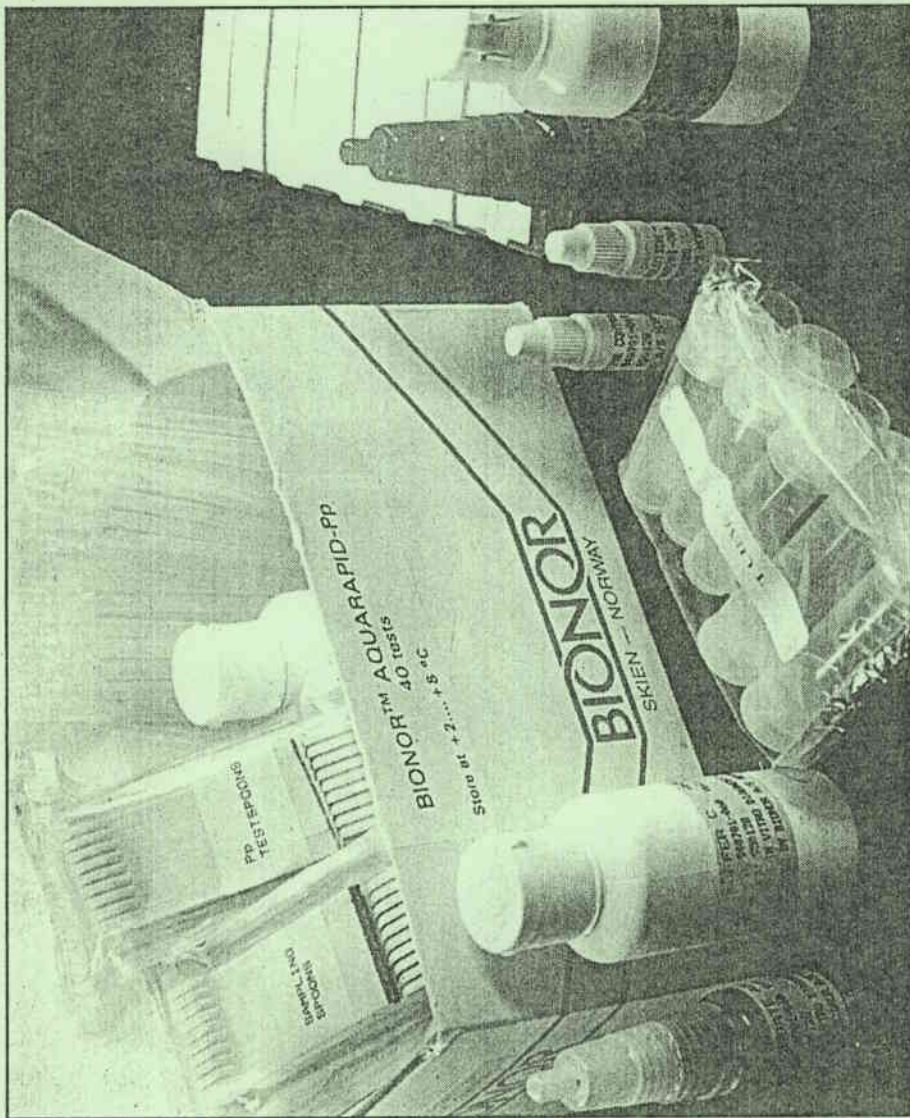
rated due to the time and effort needed to examine each sample by the classic bacteriological process, which restricts the number of samples taken.

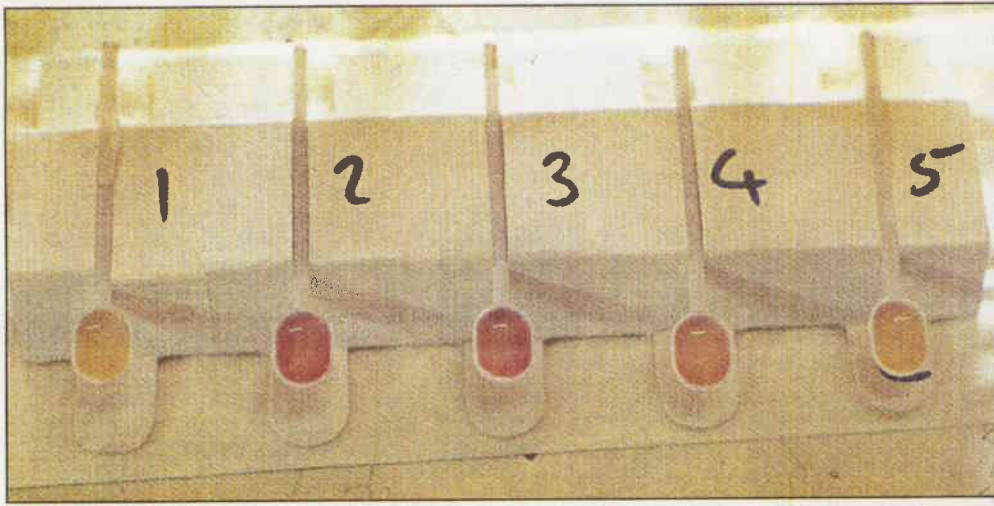
Aware of these problems, Greek fish farmers increasingly take advantage of the modern *in vitro* diagnostic kits which have recently become available to them.

These comprise the Aquarapid range of kits manufactured in Norway by Bionor A/S, which include the Aquarapid-Va testing for *Vibrio anguillarum* caus-

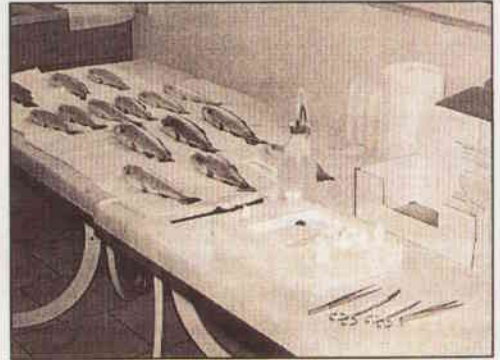
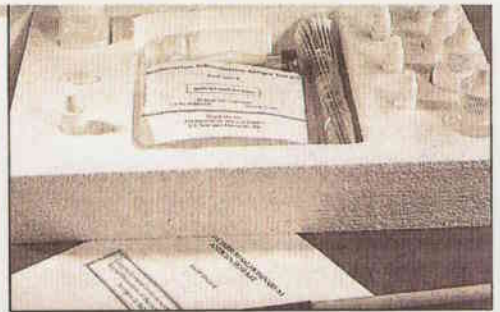
ing vibriosis in salmonids, bass and bream as well as eels; the Aquarapid-Pp for *Pasteurella piscicida* causing pasteurellosis also in bass and bream, the Aquarapid-Yr for *Yersinia ruckeri*, the causative agent of yersiniosis or enteric red mouth disease (ERM) in salmon and trout; and the Aquarapid-As for *Aeromonas salmonicida* causing furunculosis in salmon and trout.

Also available in the Greece are the KwiK-Direct and K-Dtect kits, manufac-





Left: Distinct colour change depicts the positive samples and compares the positive control (No. 5). Colour intensity relates to the amount of bacterial antigen present in the sample.



Top right: The Kwik-DTect kit tests 50 samples against the causative bacterium of BKD disease in salmonids.

Right: The diagnostic ready to be applied to a number of fish.

tured by Diag-Xotics Inc. of the USA, testing for *Renibacterium salmoninarum*, which is the causative bacterium of BKD in salmonids.

The kits provide in a self-contained package all materials and reagents needed to sample and quickly test a large number of dead, moribund or even seemingly healthy fish, be they tiny larvae, fry, or larger specimens. The testing process follows a specific procedure set out in easy-to-follow user instructions and the results are read within one to three hours.

These become visible by the eventual colour change of the positive samples after comparing all samples with the enclosed positive and negative controls. The intensity of the colour change is directly proportional to the degree of infection of the particular sample.

Sensitive

The tests utilise the principles of the ELISA (Enzyme Linked Immunosorbent Assay) technique with no margin for error. Continuous stringent field and laboratory testing of these kits in different locations has not documented any false positive reactions.

Their sensitivity is remarkable. In field trials on several Greek farms, they have proved adequately sensitive with sampled tissue in the range 0.1 to 0.2 gram. This may be crucial when testing tiny fish larvae. These may be squashed/homogenised and then sampled; or with those that are suitably large, a target organ may be isolated and used, for example, the minute spleen of a one gram sea bream larva. With larger fish, parts of an organ, usually the kidney, or material from an external lesion, may be easily sampled.

The benefits are clear: disease symptoms on the very small larvae are not obvious and rapid diagnosis followed by appropriate treatment may control an imminent disease outbreak and avoid high mortalities, which in the case of acute pasteurellosis in bream may rise to more than 50 per cent among 50-60 day old fish. Several days would be needed to obtain the results of a classic bacteriological analysis, by which time mortality could not be avoided.

Direct detection of bacterial antigens from fish tis-

suages with diagnostic kits is convenient for on-site testing of diseased fish at room temperature.

Screening an entire fish population against certain diseases is thus made a practical possibility. The diagnostics are straightforward in their application. They do not need equipped laboratories or professional skills, although it is highly recommended that the interpretation of the results, and in particular the reflections to the health status of whole fish populations should be carefully assessed after sound random sampling.

Each kit usually provides for 40 or 50 sample tests, depending on type and make, but several kits may be used at any one time.

Therefore, as large a number of samples as required may be tested against more than one disease in a matter of hours.

Some of the range of applications from which Greek fish farmers have benefited include rapid detection of pathogens prior to disease outbreaks and/or the application of prophylactic antibiotic treatment or vaccination (detection of carriers); quality control and assessment of the likely stress-related mortalities before transport of fingerlings; general disease and quality control of growing fish stocks; and differential diagnosis of diseases producing confusing external symptoms and/or internal signs.

It is expected that the more widespread routine application of these kits will enhance the disease awareness among Greek farm and hatchery managers and lead further to the routine screening of eggs prior to hatching; environmental screening, such as of holding tank sediments; and prevention of potential importation of harmful diseases through infected eggs or fry.

These diagnostic tools ultimately alleviate guesswork and much of the uncertainty from the day-to-day stock health management. Early diagnosis of disease carriers, especially when used alongside a well defined fish vaccination policy, is expected to reduce antibiotic use, prevent mortalities and improve the quality of the fish and the profits of the farm.

The diagnostic kits mentioned above are available in Greece from Vet Care Ltd, 19 Dimitressa Street, 115 28 Athens. Tel/Fax: +30 1 729 4348.

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